(4)

DNA-TR-88-4

PROSTAGLANDINS AND RADIATION ENTERITIS

J. P. Delaney
University of Minnesota
Department of Surgery
Medical School
Phillips-Wangensteen Building
516 Delaware Street S.E.
Minneapolis, MN 55455

30 December 1987

Technical Report

CONTRACT No. DNA 001-85-C-0001

Approved for public release;
distribution is unlimited.

THIS WORK WAS SPONSORED BY THE DEFENSE NUCLEAR AGENCY
UNDER RDT&E RMC CODE B35108566 M C 00001 25904D

Prepared for
Director
DEFENSE NUCLEAR AGENCY
Washington, DC 20305-1000

Destroy this report when it is no longer needed. Do not return to sender.

PLEASE NOTIFY THE DEFENSE NUCLEAR AGENCY ATTN: TITL, WASHINGTON, DC 20305 1000, IF YOUR ADDRESS IS INCORRECT, IF YOU WISH IT DELETED FROM THE DISTRIBUTION LIST, OR IF THE ADDRESSEE IS NO LONGER EMPLOYED BY YOUR ORGANIZATION.

CUT HERE AND RETURN

DISTRIBUTION LIST UPDATE

This mailer is provided to enable DNA to maintain current distribution lists for reports. We would appreciate your providing the requested information.

☐ Add the individual listed to your distribution list.

☐ Delete the cited organization/individual.

☐ Change of address.

NAME: _____

ORGANIZATION: _____

OLD ADDRESS	CURRENT ADDRESS
_____	_____
_____	_____
_____	_____

TELEPHONE NUMBER: (___) _____

SUBJECT AREA(s) OF INTEREST:

_____	_____
_____	_____
_____	_____

DNA OR OTHER GOVERNMENT CONTRACT NUMBER: _____

CERTIFICATION OF NEED-TO-KNOW BY GOVERNMENT SPONSOR (if other than DNA):

SPONSORING ORGANIZATION: _____

CONTRACTING OFFICER OR REPRESENTATIVE: _____

SIGNATURE: _____

Director
Defense Nuclear Agency
ATTN: TITL
Washington, DC 20305-1000

Director
Defense Nuclear Agency
ATTN: TITL
Washington, DC 20305-1000

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

AD-A199 150

REPORT DOCUMENTATION PAGE

1a REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b RESTRICTIVE MARKINGS
2a SECURITY CLASSIFICATION AUTHORITY N/A since Unclassified	3 DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b DECLASSIFICATION/DOWNGRADING SCHEDULE N/A since Unclassified	
4 PERFORMING ORGANIZATION REPORT NUMBER(S)	5 MONITORING ORGANIZATION REPORT NUMBER(S) DNA-TR-88-4
6a NAME OF PERFORMING ORGANIZATION University of Minnesota, Dept. of Surgery, Medical School 6b OFFICE SYMBOL (If applicable)	7a NAME OF MONITORING ORGANIZATION Director Defense Nuclear Agency
6c ADDRESS (City, State, and ZIP Code) Phillips-Wangensteen Building 516 Delaware Street S.E. Minneapolis, MN 55455	7b ADDRESS (City, State, and ZIP Code) Washington, DC 20305-1000
8a NAME OF FUNDING/SPONSORING ORGANIZATION 8b OFFICE SYMBOL (If applicable) AFRRI/Luckett	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DNA 001-85-C-0001
9c ADDRESS (City, State, and ZIP Code)	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 62715H; PROJECT NO: M; TASK NO: C; WORK UNIT ACCESSION NO: DH008549

11 TITLE (Include Security Classification)

PROSTAGLANDINS AND RADIATION ENTERITIS

12 PERSONAL AUTHOR(S)
Delaney, John P.

13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Technical	FROM 850501 TO 870430	871230	86

16 SUPPLEMENTARY NOTATION
This work was sponsored by the Defense Nuclear Agency under RDT&E RMC Code B35108566 M C 00001 25904D.

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Prostaglandins Radiation Enteritis Rat Model
6	5		
6	1		

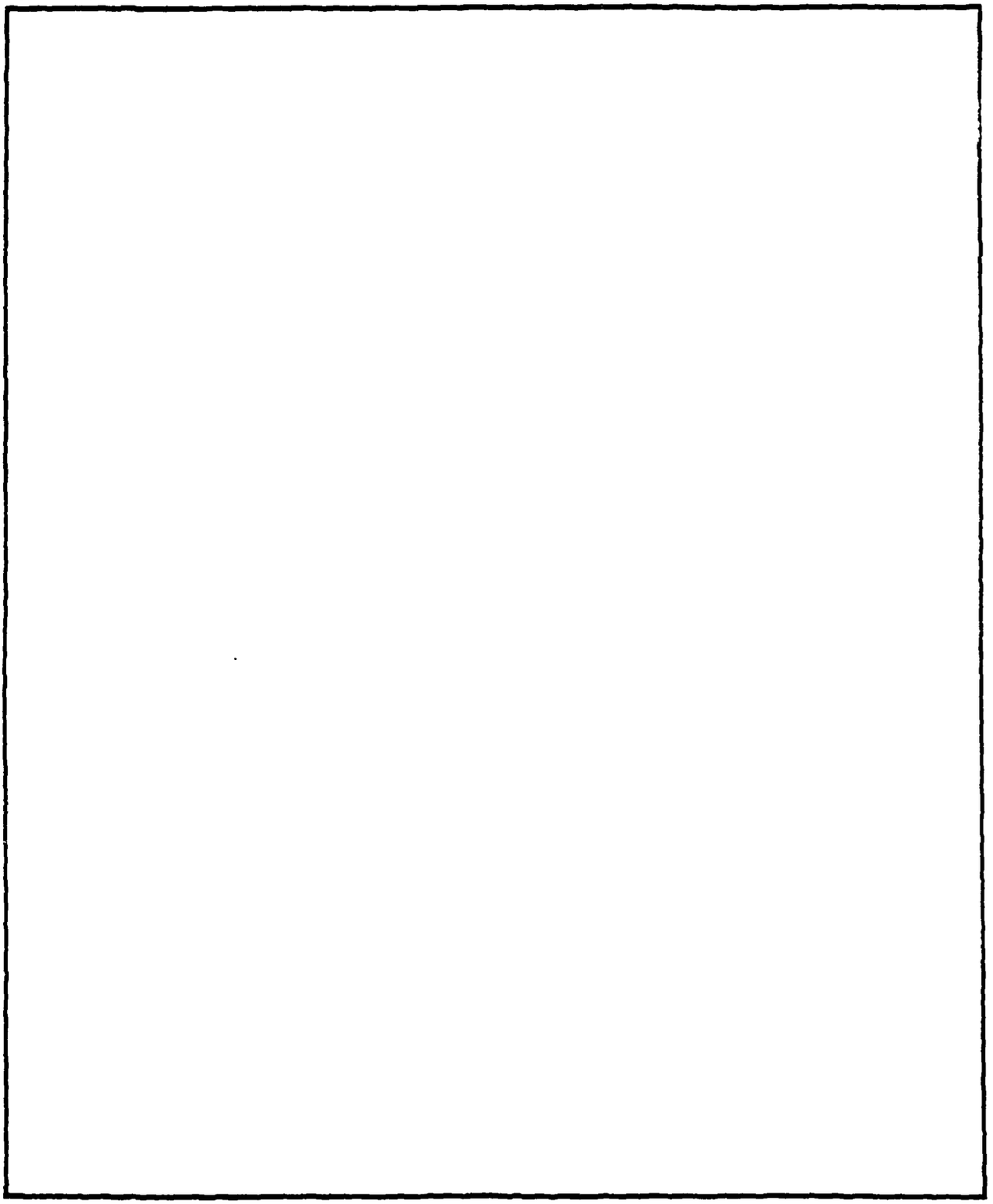
19 ABSTRACT (Continue on reverse if necessary and identify by block number)

A series of experiments was done to test the proposition that radiation to the intestine induces prostaglandin production which in turn enhances the radiation injury. The results did not support the hypothesis, in that, pharmacologic blockade of prostaglandins or pretreatment with systemic prostaglandins did not significantly alter mortality following abdominal radiation. Neither did prostaglandin manipulations alter hemopoietic suppression. Enteropolling was not induced by abdominal radiation. Systemic pretreatment with free radical inhibitors did not alter mortality following abdominal radiation. Intestinal mucosal integrity was found to be partially preserved by a variety of agents placed in the lumen prior to radiation of isolated intestinal segments. These included prostaglandin E_2, cyclooxygenase inhibitors, and adrenal cortical steroids.

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	UNCLASSIFIED
22a NAME OF RESPONSIBLE INDIVIDUAL Sandra E. Young	22b TELEPHONE (Include Area Code) (202) 325-7042 22c OFFICE SYMBOL DNA/CSTI

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED



SUMMARY

This project was designed to test the potential effects of prostaglandins on radiation injury to the intestine. The basic initial observation was that pretreatment with prostaglandin inhibiting drugs did not alter mortality patterns following abdominal radiation. Premedication with systemic prostaglandins also resulted in little effect on mortality patterns following abdominal radiation although there was a trend suggesting a slight survival benefit. Prostaglandin blockade prior to whole body or abdominal radiation did not alter the pattern of subsequent leucopenia or recovery there from. Secretion of prostaglandins was diminished following radiation to the whole abdomen. However, when exteriorized intestine was radiated, prostaglandin excretion was not significantly altered. We concluded that radiation to the entire abdomen possibly influenced renal function to result in reduced prostaglandin excretion. Dietary depletion of prostaglandin production by means of a fatty acid deficient diet did provide significant survival advantage following abdominal radiation. Abdominal radiation did not result in outpouring of fluid into the intestinal lumen (enteropooling) nor did it cause a change in the water content of the intestinal wall. Oral prostaglandin administration in the same system did cause enteropooling.

A number of studies were done in addition to the those outlined in the contract proposal. These involved pretreatment with agents which inhibit the generation of free radicals. Allopurinol, DMSO, iron depletion, and premedication with superoxide dismutase plus catalase did not alter mortality patterns following radiation. Dazmagrel, a thromboxane synthetase inhibitor had no effect. Oral administration of Trasylol, an antiprotease trypsin inhibitor, and cholestyramine, a bile salt binder, similarly failed to alter mortality following abdominal radiation. Reduction of gut flora by oral antibiotics slightly improved mortality. Premedication with quinacrine, a phospholipase A inhibitor, did show a modest protective effect. Glucocorticoid pretreatment yielded a minimal survival advantage as well.

An original method was developed to test lumenal interventions in exteriorized isolated intestinal segments. The end point was histologic evaluation of intestinal mucosal damage, using mucosal height, mucus cell recovery, inflammation, and vascularity as the parameters. In this series of experiments there were a number of positive observations. That is, a number of agents placed in the lumen at the time of the radiation proved to yield significant mucosal protection. These included PGE_2, indomethacin, aspirin, Trasylol, DMSO, and cholestyramine. Negative results were observed with lumenal Allopurinol and superoxide dismutase.

PREFACE

Animal Experimentation. Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council.

Views presented in this report are those of the author. No endorsement by the Defense Nuclear Agency has been given or should be inferred.

CONVERSION TABLE

Conversion factors for U.S. Customary to metric (SI) units of measurement

MULTIPLY → BY → TO GET TO GET ← BY ← DIVIDE		
angstrom	1.000 000 X E -10	meters (m)
atmosphere (normal)	1.013 25 X E +2	kilo pascal (kPa)
bar	1.000 000 X E +2	kilo pascal (kPa)
barn	1.000 000 X E -28	meter^2 (m^2)
British thermal unit (thermochemical)	1.054 350 X E +3	joule (J)
calorie (thermochemical)	4.184 000	joule (J)
cal (thermochemical)/cm^2	4.184 000 X E -2	mega joule/m^2 (MJ/m^2)
curie	3.700 000 X E +1	*giga becquerel (GBq)
degree (angle)	1.745 329 X E -2	radian (rad)
degree Fahrenheit	$t_k = (t°f + 459.67)/1.8$	degree kelvin (K)
electron volt	1.602 19 X E -19	joule (J)
erg	1.000 000 X E -7	joule (J)
erg/second	1.000 000 X E -7	watt (W)
foot	3.048 000 X E -1	meter (m)
foot-pound-force	1.355 818	joule (J)
gallon (U.S. liquid)	3.785 412 X E -3	meter^3 (m^3)
inch	2.540 000 X E -2	meter (m)
jerk	1.000 000 X E +9	joule (J)
joule/kilogram (J/kg) (radiation dose absorbed)	1.000 000	Gray (Gy)
kilotons	4.183	terajoules
kip (1000 lbf)	4.448 222 X E +3	newton (N)
kip/inch^2 (ksi)	6 894 757 X E +3	kilo pascal (kPa)
ktap	1.000 000 X E +2	newton-second/m^2 ($N-s/m^2$)
micron	1 000 000 X E -6	meter (m)
mil	2.540 000 X E -5	meter (m)
mile (international)	1.609 344 X E +3	meter (m)
ounce	2.834 952 X E -2	kilogram (kg)
pound-force (lbs avoirdupois)	4.448 222	newton (N)
pound-force inch	1.129 848 X E -1	newton-meter (N·m)
pound-force/inch	1.751 268 X E +2	newton/meter (N/m)
pound-force/foot^2	4.788 026 X E -2	kilo pascal (kPa)
pound-force/inch^2 (psi)	6.894 757	kilo pascal (kPa)
pound-mass (lbm avoirdupois)	4.535 924 X E -1	kilogram (kg)
pound-mass-foot^2 (moment of inertia)	4.214 011 X E -2	kilogram-meter^2 ($kg·m^2$)
pound-mass/foot^3	1.601 846 X E +1	kilogram/meter^3 (kg/m^3)
rad (radiation dose absorbed)	1.000 000 X E -2	**Gray (Gy)
roentgen	2.579 760 X E -4	coulomb/kilogram (C/kg)
shake	1.000 000 X E -8	second (s)
slug	1.459 390 X E +1	kilogram (kg)
torr (mm Hg, 0° C)	1.333 22 X E -1	kilo pascal (kPa)

*The becquerel (Bq) is the SI unit of radioactivity; 1 Bq = 1 event/s.
**The Gray (Gy) is the SI unit of absorbed radiation.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF TABLES (CONCLUDED)

SECTION 1

INTRODUCTION

This report is written in sections. The introduction and methods sections are self-explanatory. The section on preliminary studies outlines the experiments done in this laboratory prior to submission of the original proposal. Section 4 follows the same order as the original request, and is titled Contract Studies. Section 5 describes additional survival studies not included in the original request. Finally, Section 6 outlines experiments involving radiation of isolated intestine.

Reported experiments which demonstrated protection of the esophagus against radiation injury by means of anti-inflammatory agents stimulated us to test the possibility that the small intestine might be protected similarly. An initial series of studies using indomethacin pretreatment resulted in a marked diminution in mortality following abdominal radiation. From this observation we developed a hypothesis regarding the importance of prostaglandins in the pathogenesis of radiation enteritis. An underlying speculation was that prostaglandins and free radicals might somehow interact in radiation damage to intestinal mucosa.

A number of technical aspects must be considered for the reader to understand the details of the experiments described in this text. The first is that we employed *abdominal* radiation. This is to be contrasted with the majority of previous studies in which whole body radiation was used to study intestinal damage. Our idea was that whole body radiation has a number of other consequences which may interfere with survival by means unrelated to intestinal injury. The intent of this series of studies was to concentrate on intestinal injury alone. For example, hemopoietic depression was much less severe than one would have anticipated with similar doses of radiation to the whole body. Thus, the animals were not dying of unrelated septic complications. Certainly, radiation to the whole abdomen does result in damage to organs other than the intestine. This issue was further addressed in later studies on isolated intestine.

The chosen end point was death. In a few instances, particularly with indomethacin, where we believed protection was evident, other aspects of the post radiation syndrome were studied, such as hematopoietic depression, sequential histological changes in the mucosa, and prostaglandin excretion in the urine. Numerous biochemical parameters were also observed following abdominal radiation with and without indomethacin pretreatment.

In later studies we were unable to reproduce the apparent indomethacin protection. Many efforts were made to explain the discrepancies between initial studies and the later ones. All our attempts were unsuccessful and we remain mystified as to these differing results.

General technical aspects to be considered relate to the delivery of the abdominal radiation. Initially the experiments were done with a 4MEV linear accelerator. Because this same machine was used for patient treatment, scheduling was extremely undependable. We therefore switched to the use of a

machine dedicated to experimental studies, a General Electric Maximar 220 KV x-ray apparatus. During the course of experiments we added a copper filter to reduce scatter. Another variable in the radiation technique involved the size of the field. Early we used a 4.0 x 5.5 cm field. Later we switched to a 4.0 x 8.0 cm field. This required dosage adjustments to obtain the approximate 80% mortality we sought in the control groups. In reviewing the results of these experiments the reader will find differences in control group mortality based largely upon technique changes. It should be emphasized that every experiment included a concurrent control group. Thus, the comparisons were valid in spite of technical changes. An additional problem arose because the tube in the Maximar machine required replacement on two occasions. Thus three different x-ray tubes were used. Each time a new tube was installed we recalibrated the machine and did the control experiments to determine the appropriate dosage of radiation to induce the desired mortality curve.

When it became evident that the nonsteroidal anti-inflammatory agents were not making a significant change in post radiation mortality, we tested a variety of other agents thought to be potentially relevant to intestinal injury. Prostaglandins were administered with the idea that if prostaglandin blockade might improve survival then prostaglandin excess would have the opposite effect. This did not prove to be the case.

The most significant observation emanating from the contract proposal was that dietary depletion of prostaglandins by means of an essential fatty acid deficient diet did provide a measure of radioprotection as judged by survival following abdominal radiation.

We then studied a series of potentially influential pharmacologic agents and describe those experiments under "Additional Survival Studies". The drugs included some which interfere with free radical production. In the intestine Allopurinol blocks reactions leading to free radical release and has been shown to be protective against ischemia-reperfusion and a variety of other insults. It did not prove to be protective against radiation injury. Along similar lines, desoxyferamine was used to deplete iron availability and thus presumably inhibit enzymatic reactions needed for free radical generation. This too failed to alter mortality patterns. Dimethyl sulfoxide is a free radical scavenger. Pretreatment with dimethyl sulfoxide failed to alter mortality. Superoxide dismutase and catalase also interfere with free radical damage in a number of experimental systems. Premedication with these agents similarly failed to provide protection against death after abdominal radiation.

Synthetic adrenal cortical steroids are said to stabilize membranes and thus might interfere with generation of prostaglandins from membrane arachidonic acid. There was a suggestion that steroid treatment did yield a small survival advantage.

We have previously shown that depletion of bile or pancreatic juice from isolated segments of intestine by operative rearrangements lead to diminished histologic injury after radiation. We therefore tested oral administration of Trasylol, a proteinase inhibitor, and the oral administration of cholestyramine, a bile salt binder. Neither significantly altered post radiation mortality.

At this juncture, using mortality as an end point and systemic administration of drugs, we had not observed significant protection with prostaglandin excess, prostaglandin depletion, free radical inhibition, or luminal inactivation of bile salts and proteolytic enzymes.

The third group of studies differed markedly from the others in three regards. A portion of the intestine was operatively exteriorized and subjected to radiation comparable to the dose which had proved lethal when delivered to the whole abdomen. The animals invariably survived, and therefore it was possible to observe histologic changes in the radiated intestine at various time intervals after the insult. The second difference was that the end point studied was not mortality but mucosal histology.

The third fundamental difference from previous experiments was that these studies related to topical effects of agents bathing the mucosa from the lumenal side. No systemic drugs were given except the anesthetic. A particular advantage to this approach is the ease of establishing control values. In a single animal we were able to compare unradiated intestine, radiated intestine containing the agent in question, and radiated intestine containing only saline. In this fashion each animal served as its own control.

As a generalization, results of these latter experiments have proved to be far more provocative and promising than those described in the original contract proposal. Our methodology has been criticized because of the histologic parameters chosen, specifically mucosal thickness, mucus cell depletion-restoration, inflammation, and vascularity. Critics have proposed that we would gain more valuable information by quantitating crypt cell survival. We have repeatedly attempted to make such measurements but have been unsuccessful. The damage in this model is such that crypt cells are unidentifiable at the peak of the injury. Others have observed too that crypt cell quantitation in the rat is not satisfactory, as contrasted with the mouse where it is readily done and readily duplicated. We found that mucosal height was an excellent reflection of injury, that reduction of mucosal height followed a perfectly predictable pattern and that restoration of mucosal histological normality also was stereotypical. Mucus cell changes closely parallelled the mucosal thickness changes. Inflammation and vascularity were useful but less objective and probably less discriminating measures of injury.

Using this model we observed some mucosal preservation when prostaglandins were present in the lumen during radiation. Paradoxically there seemed to be benefit from Indocin and from aspirin in the lumen. Both cholestyramine and Trasylol also provided histologic protection. Similarly luminal steroids, in very few experiments, provided preservation of mucosal height. The free radical scavenger, superoxide dismutase, in the lumen yielded no protection.

From our perspective, the most useful development to come out of this series of studies is a new method for radiating exteriorized segments of intestine during lumenal interventions. The method should lend itself to further studies aimed ultimately at oral administration of agents for pretreatment before anticipated intestinal radiation. This approach would have particular value for military application in that the potential victim of radiation injury could carry an agent which taken orally would serve to diminish injury to the intestine,

and thus diminish the early radiation effects, the intestinal syndrome. With such protection the effectiveness of the individual could be maintained even though otherwise incapacitating radiation exposure had been sustained.

The initial group of studies was modestly rewarding in a negative sense. Reasonable hypotheses were outlined but subsequently disproved. Later studies exploring intraluminal influences on radiation injury, seemed to be most promising and potentially of practical value.

1.1 BACKGROUND FOR CONTRACT STUDIES

Radiation to whole body or to the abdomen leads to acute intestinal injury which is manifested as the "intestinal syndrome". Early death after excessive intestinal radiation follows a characteristic pattern. Survival time after a single dose of radiation sufficient to cause acute intestinal death is quite constant within a species and is relatively independent of radiation dose.[10,20] That is, if enough radiation is given to be fatal, death occurs at a predictable time interval after the insult. The target organ is the small intestine and the fundamental pathologic change is loss of the epithelial lining.[20,31] The threshold dose for intestinal death approximates 1000 rads[10] depending upon the species, the age of the animal and radiation variables.[10] Deaths which occur in the first week following radiation overdosage are directly attributable to intestinal injury and are termed "intestinal death". Survival following acute abdominal exposure to excess radiation will depend upon prevention and/or treatment of the gastrointestinal syndrome.

The victim of radiation overdose can be supported through later hematopoietic effects until the marrow recovers. However, this therapeutic possibility will not arise unless he can be tided over the early radiation effects on the intestine. The period at risk is relatively short, that is the intestinal mucosa begins to regenerate within four to six days after the insult and is sufficiently well reconstituted after fourteen days to allow survival.[1,15,28,32,44] Thus if radiation exposure can be anticipated and prepared for or if the victim can be treated for the intestinal syndrome the possibility of survival is enhanced. The implications in the combat situation are obvious. In certain circumstances the possibility of radiation exposure can be anticipated and prophylactic measures applied if and when such measures are developed. Treatment of the gastrointestinal syndrome after radiation exposure is also a possibility, albeit less promising than prophylaxis.

We proposed an original fundamental hypothesis regarding radiation injury to the intestine with important scientific and practical implications. The hypotheses we proposed were:

1. Radiation of the intestine results in increased prostaglandin synthesis.

2. Prostaglandin synthesis is a major factor in radiation induced injury of the intestine.

3. Agents which interfere with prostaglandin synthesis protect the intestine against radiation injury.

The specific goals of this study included:

1. To determine if administration of exogenous prostaglandins alter the severity of acute radiation enteritis.

2. To determine if systemic prostaglandin depletion by dietary means provides protection against radiation induced acute gastrointestinal syndrome.

3. To determine if interference with prostaglandin synthesis by pharmacologic agents alters intestinal injury and/or permits survival after otherwise lethal abdominal radiation.

4. To ascertain if radiation leads to excess fluid secretion into the intestinal lumen (enteropooling).

5. To test pharmacologic manipulation of arachidonic acid metabolism as related to hematopoietic suppression.

SECTION 2

PRELIMINARY STUDIES

2.1 INTESTINAL DEATH

2.1.1 Background

The initial background study was designed to determine in our laboratory mortality patterns from the post-radiation intestinal syndrome induced by abdominal radiation in the rat.

2.1.2 Methods

Female Sprague-Dawley rats weighing between 200-280 grams were used. The animals received intraperitoneal injections of Ketamine (80mg/kg) for sedation twenty minutes prior to irradiation. They were immobilized and a ventral abdominal port extending from xiphoid to pubis outlined. The thorax and limbs were excluded from the radiation beam by lead collimators. A 4MeV linear accelerator delivered the selected amount of radiation as a single dose calculated at the midline of the animal. The delivery rate was 320 centiGray (cGy) per minute. The source to skin distance was 80 cm. All preliminary studies in this laboratory were done with a 4MeV linear accelerator. The end point was death within 15 days.

2.1.3 Results

The animals developed typical symptoms of the intestinal syndrome, diarrhea, lethargy, ruffled coat and runny eyes. Survival data are given in table 1.

Table 1. Mean survival times for 800, 900, 1000, 1100, and 1200 cGy abdominal radiation using 4 MeV radiation.

Radiation Dose(cGy)	Number of Rats	Percent Mortality	Days to Death Range	Mean*
1200	10	100	4-7	4.5
1100	10	100	4-5	4.2
1000	12	66	4-15	8.5
900	8	75	4-5	4.5
800	5	0	-	-

*Mean survival time calculations include only those animals that died.

In this model the LD_{50} for intestinal death was somewhere between 800 and 1000 cGy to the abdomen. 1100 cGy was almost always fatal. The findings agree with those of Dunjic et al who found in similar studies that 1150 cGy to the abdomen resulted in early death in 95% of animals, 1000 cGy - 70%, 900 cGy - 45%, and 800 cGy - 10%.[10] Based on these results, we elected to use a radiation dose of 1100cGy to measure possible radioprotection.

2.2 INDOMETHACIN TOXICITY

2.2.1 Background

Indomethacin is a non-steroid anti-inflammatory compound (NOSAC) which blocks prostaglandin synthesis by inhibiting cyclooxygenase the enzyme responsible for conversion of arachidonic acid to endoperoxides, which in turn give rise to prostaglandins.

Indomethacin in high doses causes gastric and intestinal ulcerations. If the dose is sufficiently large the animals die from peritonitis.[18] In order to select a dose of the drug that would not by itself cause fatal gastrointestinal perforations we studied indomethacin toxicity.

2.2.2 Methods

On the basis of literature background we selected doses of 4, 10, 15, and 20 mg/kg body weight, injected intraperitoneally.[32] There were five animals in each group. Survivors were sacrificed at one week and the intra-abdominal contents examined.

2.2.3 Results

Four mg/kg indomethacin caused no deaths. The gastrointestinal tract was intact at sacrifice. All animals receiving 10 mg/kg survived for one week. Three of these five had perforated small bowel and peritonitis. All animals receiving 15 mg/kg died from peritonitis between the fourth and seventh day. Of the five animals receiving 20 mg/kg, two died on day four. The other three had peritonitis at sacrifice on day seven.

There was no histologic evaluation for rats that died before the one week period because of tissue autolysis, but tissue samples were taken from all rats sacrificed on day seven. Tissues from rats receiving 4 mg/kg were normal with no hemorrhage or inflammation. The intestinal tissue from rats receiving 10 mg/kg showed extensive hemorrhage in all five surviving animals. All three of the animals receiving 20 mg/kg and surviving one week showed extensive hemorrhage and some loss of mucosal height. On the basis of these results we chose 4mg/kg as a tolerable single intraperitoneal dose of indomethacin for the rat. This dose was used in subsequent studies.

2.3 INDOMETHACIN RADIOPROTECTION

2.3.1 Methods

Having established 1100 cGy of abdominal radiation from the linear accelerator as uniformly lethal and 4 mg/kg indomethacin as a tolerable dose, we gave 1100 cGy abdominal radiation to 15 rats one hour after an intraperitoneal injection of 4 mg/kg indomethacin.

Secondly, to test the effect of smaller doses of indomethacin rats received 2.0, 1.0, or 0.5 mg/kg intraperitoneally one hour prior to 1100 cGy abdominal radiation.

2.3.2 Results

Of the 15 rats receiving 4 mg/kg indomethacin, one rat died at six days. The other 14 survived until sacrifice at three weeks. The results with smaller indomethacin doses are given in table 2. These smaller doses of the drug also appeared to be effective in reducing mortality after 1100cGy abdominal radiation. This is in accord with the general observation that 1 mg/kg of indomethacin does effectively block cyclooxygenase activity in the rat. From these results, it was obvious that indomethacin provided significant protection against death from the intestinal syndrome. As noted above, 1100 cGy from the linear accelerator to the abdomen killed 95-100% of unprotected rats. This observation encouraged us to pursue the role of non-steroidal anti-inflammatory agents as radio protectants for the intestine.

Table 2. Percent mortality after indomethacin and 1100cGy abdominal radiation.

Indomethacin mg/kg	Number of Rats	Percent Mortality Within 14 Days
4.0	15	7 (1 of 15)
2.0	5	0
1.0	4	50 (2 at day 5)
0.5	5	0

2.4 OTHER EFFECTS OF INDOMETHACIN PRETREATMENT

2.4.1 Background

Although rats pretreated with indomethacin survived the insult of 1100 cGy abdominal radiation, they followed a course in the first four post radiation days similar to those animals receiving no drug. They showed signs of the intestinal syndrome, diarrhea, anorexia, lethargy, and dull, shaggy coat. They began to improve about the fourth or fifth day, while the unprotected rats proceeded to die. The next study was aimed at delineating differences between indomethacin protected and unprotected animals on the day before death.

2.4.2 Methods

Three groups of rats were included in this study. The first group was non-irradiated controls; the second group received 1100 cGy abdominal radiation; and the third group received 4mg/kg indomethacin intraperitoneally one hour prior to 1100cGy abdominal radiation. All rats were weighed prior to radiation and at the time of sacrifice.

On post radiation day 4, all rats were etherized to near death and blood was drawn by cardiac puncture for biochemical variables. Fifteen parameters were measured in the three groups. Because of severe dehydration, sufficient blood was obtained in only four indomethacin and five radiated control animals.

2.4.3 Results

Mean weight change as a percent of body weight for each of the three groups is given in table 3. By this single variable, indomethacin pretreatment provided no discernable nutritional or hydrational benefits.

Table 3. Weight change in four days as a percent of body weight.

Group	Percent Weight Change
Control	+ 1.0
1100cGy	- 18.5
Indomethacin & 1100cGy	- 16.7

Biochemical screening showed no significant differences in serum bilirubin, L.D.H., or GOT. Other observations from biochemical testing are summarized in table 4 as the mean values +/- SEM. These results are not simply interpreted. As a generalization, indomethacin pretreatment did not blunt the serum biochemical changes as compared to radiated rats receiving no drug. The notable exceptions were serum calcium and phosphorus which were both significantly depressed in the radiated control group as compared to the indomethacin pretreated group.

Table 4. Mean biochemical values for control, 1100cGy abdominal radiation, and 4mg/kg indomethacin intraperitoneally one hour prior to 1100cGy abdominal radiation.

	Control (+/-SEM)	Indomethacin & Radiation (+/-SEM)	Radiation Only (+/-SEM)
Total Protein(gm%)	6.9 (0.80)	↑8.6 (0.19)	7.2 (0.20)
Globulin (gm%)	3.4 (0.11)	↑4.3 (0.19)	3.4 (0.14)
Albumin (gm%)	3.5 (0.08)	↑4.2 (0.10)	3.8 (0.10)
Uric Acid (mg%)	3.4 (0.31)	↓2.3 (0.27)	↓2.1 (0.29)
BUN (mg%)	19.0 (0.50)	↑69.0 (10.7)	27.0 (2.90)
Creatine (mg%)	0.5 (0.02)	↑0.8 (0.05)	0.4 (0.04)
Amylase (Somogyi Units)	365.0 (87.0)	↑2722.0 (180.0)	↑1931.0 (114.0)
Phosphorus (mg%)	9.4 (0.30)	10.0	↓7.3 (0.40)
Calcium (mgg%)	11.1 (0.20)	11.1 (0.10)	↓9.9 (0.20)
Glucose (gm%)	187.0 (12.0)	↑412.0 (43.0)	229.0 (20.0)
Alkaline Phosphatase (Intestinal Units)	284.0 (30.0)	↓143.0	↓104.0 (6.00)
Cholesterol (mg%)	86.0 (5.00)	↑142.0 (8.00)	↑115.0 (6.00)

NOTE: ↑Significant increase (P<.05) from control.
↓Significant decrease (P<.05) from control.

Elevated BUN, creatinine and serum proteins in the indomethacin group likely were the result of dehydration, although, as noted above, weight loss was the same whether or not the radiated animals had been pretreated. Liver function tests were not notably altered by radiation.

Of interest was the doubling of serum amylase following radiation. This may represent leakage of the enzyme from the injured intestine or may be secondary to radiation injury of the pancreas. The elevation of blood glucose following radiation also suggests possible damage to the pancreatic islets.

SECTION 3

GENERAL METHODS

3.1 ANIMALS

Adult female Sprague-Dawley rats from BioLabs (St. Paul, MN.) weighing between 250 and 300 grams were used for these experiments. All rats were given one week of conditioning during which time they received standard lab chow and water ad lib. They were housed in a temperature and humidity controlled environment, 2 per cage, with a 12 hour light and dark cycle.

3.2 RADIATION

All of the rats in the preliminary studies were radiated with the 4 MeV linear accelerator. The use of this machine necessitated radiating the animals at the convenience of the clinical schedule. Continued use of the linear accelerator would have resulted in much loss of time. We therefore opted to use a General Electric Maximar X-ray machine dedicated to research work for the subsequent contracted studies.

Animals were sedated with 80mg/kg intraperitoneal Ketalar, positioned supine and radiated three at a time with a single dose. Lead shielding, 2mm in thickness was placed to block radiation above the xiphoid, below the pubis and between the rats. Radiation was delivered by a 220 KVp General Electric Maximar machine at 15mA with a 0.25mm added copper filter. Animals were given a calculated single dose of abdominal radiation. Since the preliminary studies used the linear accelerator to deliver abdominal radiation, a series of control rats was later radiated with the General Electric Maximar machine to establish baseline values. Figure 1 compares the mortality curves observed by these two machines. Figure 2 shows the set up for abdominal radiation.

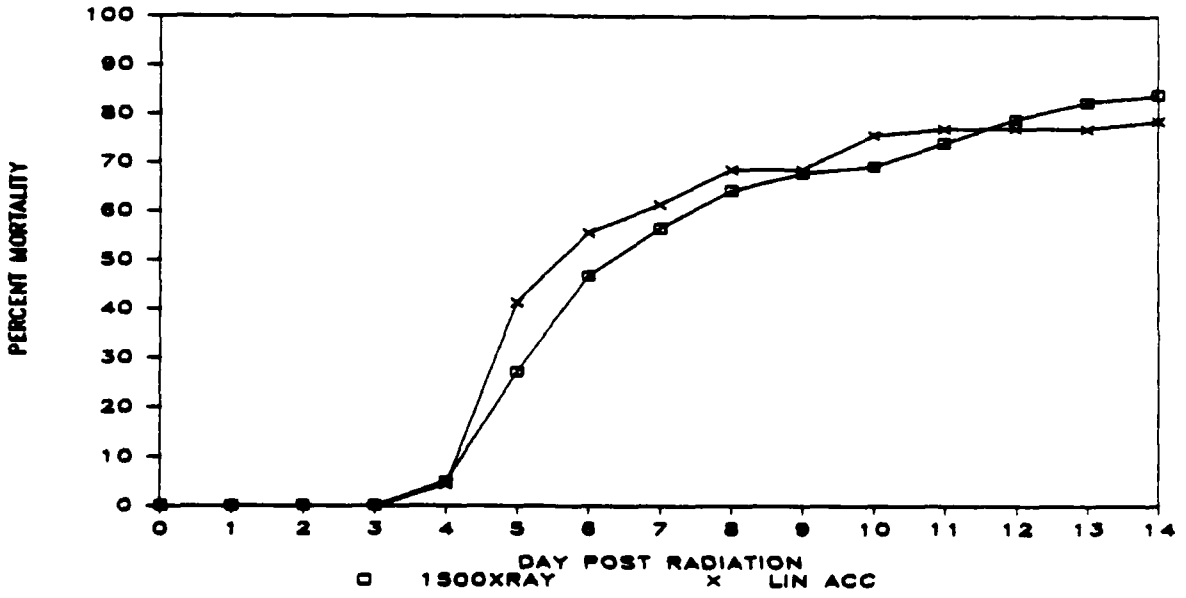


Figure 1. Percent mortality for 1100cGy linear accelerator (N=70) and 1500cGy x-ray abdominal radiation (N=143).

Figure 2. Photograph of radiation set up for abdominal X irradiation.

To verify the calculated dose rate in the rat from the Maximar machine, throw away thermoluminescent dosimeter (TLD) capsules supplied by Radiation Detection Company, Sunnyvale, CA, were utilized. The TLD's were read on a Teledyne 7300 TLD reader at a preheating temperature of 130 degrees Centigrade, a maximum reading temperature of 290 degrees Centigrade and heating amp cycle time of fifteen seconds.

Four sets of TLDs were used. One set was used to find the calibration factor, centiGray per micro Coulomb (cGy/uC) and the other three were used to determine the dose on the skin, midplane, and back of the radiated rats. The calibration TLDs were placed at a nominal focus-to-skin distance of 42.6cm to provide complete scatter contribution. A calibration factor of 1.129 cGy/uC was measured. Using this factor, the average measured dose rate at the center of the rats was 101.2 cGy/minute, plus or minus 10% which was in very good agreement with the calculated dose rate of 105.9 cGy/minute.

After radiation, animals were allowed to recover from the anesthetic and were returned to their cages. For most of the experiments rats were fasted neither before nor after radiation and were allowed water and standard lab chow ad lib.

It should be noted here, that at three different times during these studies, the x-ray tube in the Maximar machine required replacement. With each new tube, we repeated a series of control abdominal radiation studies and the corresponding TLD's. Because of the variation from tube to tube, it was sometimes necessary to change the dose of abdominal radiation needed to obtain 80 percent mortality. It also became extremely important to radiate a group of control rats at the same time each group of drug treated rats was radiated. Table 5 gives the mortality curves we obtained for each of the three x-ray tubes in the General Electric Maximar machine.

Table 5. Percent mortality for control rats for three different x-ray tubes in the General Electric Maximar machine.

cm Field	4 x 5.5	4 x 5.5	4 x 8
	1500cGy	1500cGy	1200cGy
Day After	Tube 1	Tube 2	Tube 3
Radiation	n=105	n=96	n=44
1	0	0	0
2	0	0	0
3	0	0	0
4	4	4	14
5	23	30	70
6	39	58	84
7	48	73	84
8	57	77	86
9	59	77	86
10	62	81	86
11	70	81	86
12	73	83	86
13	77	84	86
14	79	85	86

In some studies the field size was 4 x 4.5cm. In later studies, it was 4 x 8.0cm. Larger x-ray doses were required to obtain the desired mortality with the smaller field, as expected. These changes in technique were of no consequence in data interpretation because every experiment included a simultaneous control group.

3.3 TISSUE EVALUATION

In those studies involving histologic evaluation of mucosa, rats were killed by ether overdose. Segments of intestine were quickly excised and opened along the mesenteric border. Intraluminal contents were washed from the mucosal surface with saline. The opened segment of bowel was pinned flat, mucosal side up, on a piece of cork. The specimens were fixed for 48 hours in 10% neutral buffered formalin. Samples were dehydrated, cleared in xylene, and infiltrated with paraffin embedding compound in an auto-technicon. After completion of the embedding process, tissue sections were cut five microns in thickness with a microtome and mounted on glass slides, dried over night and then deparaffinized and hydrated. They were stained with hematoxylin and eosin (H&E) and with Periodic Acid-Schiff's (PAS), dehydrated, cleared, and coverslipped with Permount. PAS sections were used to identify mucus containing cells.

Tissues were scored for injury according to criteria we have previously described.[24] Each slide was evaluated with the microscopist unaware of the origin of the tissue. The following measurements were made: 1) mucosal thickness, 2)submucosal thickness, and 3)muscle thickness. The latter two proved not useful for assessing the severity of injury. These measurements were made using an eyepiece micrometer at a magnification of 100X. In addition, tissues were scored qualitatively for inflammatory infiltrate, vascular engorgement, and loss of mucus cells 0 to 4. Mucosal thickness was measured at five representative sites where the villi were visible from base to tip.

Sections from normal control rats were measured to provide normal values for mucosal height, submucosal thickness and muscle thickness. Experimental samples were compared to the normals. Inflammation score was based on an estimate of polymorphonuclear leucocyte infiltration of the bowel wall: 0=none, 1+=infiltration confined to the mucosa, 2+=mucosa plus minimal submucosal infiltrate, 3+=PMN aggregates in submucosa, and 4+=muscularis propria infiltration.

Vascularity was evaluated by estimating the degree of filling of blood vessels and by erythrocyte escape from the vessels: 0=normal control, 1+=dilation of vessels with erythrocyte stasis, 2+=erythrocyte diapedesis, 3+=local interstitial hemorrhage, and 4+=extensive areas of hemorrhage.

Mucus cell loss was evaluated on PAS-stained sections. In control intestine, mucus-containing cells were found from the base of the crypts to the tips of the villi. With progressive damage, mucus cells were lost from the villi. Scoring was as follows: 0=mucus cells present in the full length of the villi, 1+=mucus cells present in 75% of length, 2+=mucus cells present in 50% of length, 3+=mucus cells present in 25% of length, and 4+=no mucus cells present.

SECTION 4

CONTRACT STUDIES

4.1 PROSTAGLANDINS

4.1.1 Background

Prostaglandins are a widely distributed family of saturated oxygenated fatty acids derived from arachidonic acid, a cell membrane constituent. Cyclooxygenase is the enzyme responsible for transformation of arachidonic acid to endo-peroxides which subsequently are converted to prostaglandins.

Non-steroid anti-inflammatory compounds (NOSAC), such as aspirin and indomethacin block the activity of cyclooxygenase and thus inhibit the generation of prostaglandins.

While the actions of prostaglandins in stomach pathophysiology have been extensively investigated,[33] small bowel effects have received somewhat less attention.[34] One well established observation is that high doses of prostaglandins induce diarrhea.[1,34] The diarrhea is caused by excessive secretion of fluid into the small intestinal lumen, a process which has been termed "enteropooling". Other prostaglandin effects on gut function which may play some role in the radiation induced intestinal syndrome include mesenteric vasodilation,[9,44] increased intestinal oxygen consumption,[2,29] increased intestinal motility,[3] and the cytoprotective action of prostaglandins observed in the stomach.[33]

For these studies, we used the prostaglandins which have been most extensively studied with respect to stomach cytoprotection: 16,16-dimethyl-PGE_2 and PGE_2.[33] These drugs were obtained from Andre Robert of Upjohn Company who suggested appropriate dosages in the effective but non-toxic range, and from Sigma Chemical Company, St. Louis.

4.1.2 Methods

Prostaglandin E_2 was obtained from Sigma Chemical, St. Louis, and mixed in normal saline at a concentration of 100ug/ml. 16, 16-dimethyl-Prostaglandin E_2 was obtained from Upjohn Diagnostics, Kalamazoo, MI. It is an oil which is provided as a stock solution in triacetin at a concentration of 2.2 mg of prostaglandin per ml of triacetin. Upjohn suggested diluting aliquots of the stock solution in at least 15 parts of distilled water followed by vigorous agitation for five minutes.

In personal communication with other investigators (Hanson, et al)it was suggested that 16, 16-dimethyl-PGE_2 be mixed in a vehicle of normal saline with 4% ethanol because there was some question at to the activity of this drug when mixed in water. We therefore, completed later studies using the alcohol vehicle.

Three different mortality studies were completed using Prostaglandin E_2. In the first study, 250ug/kg was administered intraperitoneally to 10 rats sixty minutes prior to and 24 hours after 1340cGy abdominal X radiation from tube #1. Another group of 10 rats received 250ug/kg subcutaneously sixty minutes prior to 1340cGy abdominal radiation, but no drug 24 hours later. This study aimed to show the effects of different drug administration protocols.

The next study involved a new x-ray tube. Subcutaneous PGE_2 250ug/kg was given sixty minutes before 1500cGy abdominal radiation. The high dose of radiation, 1500cGy instead of 1340cGy was used because the new tube did not give the expected mortality at 1340cGy.

In the third study, 250ug/kg PGE_2 either subcutaneously or intraperitoneally was given sixty minutes before 1100cGy abdominal radiation from Maximar tube #3 to again look for any change in mortality due to variations in drug administration.

Control rats received the same volume of saline or alcohol by the same route. The number of surviving rats was counted each post radiation day. 16, 16-dimethyl-PGE_2 is a synthetic analog which has a much longer biologic half life.[25,26,42] It was studied in three separate survival experiments. First, 770ug/kg in saline was given subcutaneously one hour before and 24 hours after 1340cGy abdominal radiation from the Maximar tube #1. Control rats received the same volume of saline prior to radiation. In the second study, 16, 16-dimethyl-PGE_2, 770ug/kg in saline was given subcutaneously one hour prior to 1100 cGy abdominal x radiation from tube #3.

For the third study, 16, 16-dimethyl-PGE_2 was mixed with a saline solution containing 4% absolute ethanol and injected subcutaneously at a dose of either 770ug/kg or 3060ug/kg 30 minutes prior to radiation. This experiment was to determine possible differences based on alcohol versus saline as a vehicle. For this study, we changed the dose of radiation to 1200cGy from the Maximar machine because only 55 percent of the control rats had died in the second study.

4.1.3 Results

a. Prostaglandin E_2. The intraperitoneal or subcutaneous administration of 250ug/kg Prostaglandin E_2 sixty minutes prior to abdominal radiation, or subcutaneously sixty minutes prior to and 24 hours after 1340 cGy abdominal radiation did not significantly alter the mortality. All rats in the first study died by day six. Control rats in the second study showed a 20 percent greater mortality than rats receiving subcutaneous PGE_2 prior to 1500cGy abdominal radiation, but the difference was not significant.

In the third study, 250ug/kg prostaglandin E_2 given subcutaneously one hour prior to 1100cGy abdominal radiation resulted in 60 percent mortality while corresponding control animals showed 80 percent mortality. This difference was again not significant. Prostaglandin E_2 given intraperitoneally, 250ug/kg, one hour prior to 1100cGy abdominal radiation from Maximar tube #3 resulted in 20 percent mortality which was significantly different from the 60 percent mortality for the corresponding control animals (P=0.08). See table 6.

Table 6. Percent mortality following doses of abdominal radiation and PGE_2, 250ug/kg subcutaneously or intraperitoneally.

	STUDY #1				STUDY #2		STUDY #3			
Days	1340cGy		1340cGy		1500cGy		1100cGy		1100cGy	
After		IP	SC Pre&Post			SC		SC		IP
Rad.	Cont	PGE	Cont	PGE	Cont	PGE	Cont	PGE	Cont	PGE
(n)	10	10	10	9	10	10	10	10	10	10
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	50	20	50	11	10	0	20	10	10	0
5	80	80	90	44	40	10	60	30	50	20
6	100	100	100	100	50	30	80	40	60	20
7	100	100	100	100	60	40	80	50	60	20
8	100	100	100	100	60	50	80	60	60	20
9	100	100	100	100	60	50	80	60	60	20
10	100	100	100	100	60	60	80	60	60	20
11	100	100	100	100	70	60	80	60	60	20
12	100	100	100	100	90	60	80	60	60	20
13	100	100	100	100	90	70	80	60	60	20
14	100	100	100	100	90	70	80	60	60	20

b. 16,16-Dimethyl Prostaglandin E_2. No difference in mortality was observed between controls and animals given 16,16-dimethyl-PGE_2 subcutaneously one hour prior to and 24 hours after 1340 cGy abdominal radiation from Maximar tube #1. All control and drug-treated rats died by day 7.

Rats given subcutaneous 770ug/kg 16,16-dimethyl-PGE_2 in saline 1 hour prior to 1100cGy abdominal radiation from Maximar tube #3 showed a mortality rate of 40 percent which was not significantly different from 55 percent mortality for corresponding control animals (P=0.39).

For study #3, the dose of radiation was changed to 1200cGy because 1100cGy had not given the desired mortality for control animals in the previous experiment. It was also at this point where we switched to the 4% ethanol vehicle.

Rats received subcutaneously either 770ug/kg or 3060ug/kg 16, 16 dimethyl-PGE_2 in saline and 4% ethanol one hour prior to 1200cGy abdominal radiation. There was no significant difference in mortality between controls(90%) and rats receiving 770ug/kg(83%). All of the rats receiving 3060ug/kg 16,16-dimethyl-PGE_2 died between day one and day five. At the dose of radiation used, animals do not usually die before day four from radiation enteritis. Some of the deaths seen in this group were probably due to the toxicity of the higher dose of 16, 16-dimethyl-PGE_2.

Table 7. Percent mortality of rats following 16,16-dimethyl PGE_2 subcutaneously or intraperitoneally 60 minutes before radiation, and 60 minutes before plus 24 hours following abdominal radiation.

	STUDY #1		STUDY #2		S T U D Y		# 3	
Drug Route	SC in SALINE		SC in Saline		SC in EtOH		SC in EtOH	
& Dose:	770ug/kg		770ug/kg		770ug/kg		3060ug/kg	
Radiation	Pre &Post							
Dose:	1340cGy		1100cGy		1200cGy		1200cGy	
Day After	16,16		16,16		16,16		16,16	
Radiation	Cont	PGE	Cont	PGE	Cont	PGE	Cont	PGE
(n)	10	10	20	20	10	9	15	10
1	0	0	0	0	0	0	0	20
2	0	0	0	0	0	17	0	60
3	0	0	0	0	0	50	0	80
4	50	0	10	10	10	67	0	80
5	90	60	50	35	70	83	80	100
6	100	90	55	40	80	83	100	100
7	100	100	55	40	80	83	100	100
8	100	100	55	40	90	83	100	100
9	100	100	55	40	90	83	100	100
10	100	100	55	40	90	83	100	100
11	100	100	55	40	90	83	100	100
12	100	100	55	40	90	83	100	100
13	100	100	55	40	90	83	100	100
14	100	100	55	40	90	83	100	100

P=.39

4.1.4 Discussion

We interpret these results to show a suggestion of protection by pre-treatment with PGE_2. While no individual study showed a major survival advantage for the prostaglandin groups, there was a favorable trend in each study. Since we completed this work others have published results showing small but real survival advantages in mice pretreated with prostaglandins and subjected to whole body radiation.[16]

4.2 INDOMETHACIN

4.2.1 Background

Based on literature background[8,10,27] and our own toxicity studies detailed in the Preliminary Studies section, we selected 4 mg/kg as a tolerable single intraperitoneal dose of indomethacin for the rat. As stated in the Methods section, we changed from the Linear Accelerator to the General Electric Maximar machine to deliver abdominal radiation due to the frequent unavailability of the Linear Accelerator. Except for the study with the minipumps, all of the indomethacin experiments involved the General Electric Maximar X ray machine.

4.2.2 Methods

Three different studies were done using indomethacin. First, indomethacin in a dose of 4mg/kg was given one hour prior to 1000, 1100, 1200, or 1340cGy abdominal radiation. Control animals for each dose of radiation received the same volume of intraperitoneal saline.

In the second study, rats were given 4mg/kg intraperitoneal indomethacin one hour prior to receiving 1100cGy abdominal radiation from the linear accelerator. Immediately after radiation, 1mg/k/day indomethacin was delivered for seven days by an intraperitoneally placed Alzet minipump. To test the toxicity of Indocin delivered by minipump, pumps were surgically placed intraperitoneally in two rats. The pumps were filled with 2ml of an Indocin solution containing 2mg/ml emptying at a rate of 240ul/day. Four days later, the animals were sacrificed with ether, the pumps removed and weighed, the abdominal cavity inspected for signs of peritonitis and perforation, and tissues taken for histology. The rats showed no outward signs of illness and upon inspection of the abdominal cavity, no sign of peritonitis or perforation was found. The actual delivery rate of the pump was determined to be 293ul/day.

To study the toxicity of chronic oral Indocin administration, nine rats received Indocin in their drinking water for seven days at the following doses: 3 rats received 5mg/kg/day, 3 rats received 10mg/kg/day, and 3 rats received 20mg/kg/day. A second group of nine rats received Indocin in the food for seven days at the same doses.

To determine urine prostaglandin excretion, rats were housed one per cage in metabolic cages and urine was collected and measured each 24 hour period. A 24-hour urine sample was obtained on day 0 and day 7 for prostaglandin assay from 2 of the rats in each group of 3, and all rats were fasted for 24 hours before sacrifice.

To evaluate possible changes in tissue water content, rats were sacrificed on day 7 with ether, the abdomen opened and inspected for perforation and peritonitis. The small intestine was tied off at the pylorus and the cecum, separated from other tissue, removed intact and weighed. Intestinal contents were squeezed into a graduated container and the intestine was reweighed. The entire intestine was then opened along the mesenteric border, rinsed in saline, and reweighed. Pieces of proximal, mid and distal intestine were removed to be processed for histology, the remaining intestine was reweighed and desiccated to dryness at 37° Centigrade to determine percent water content.

An additional group of 10 rats received Indocin 20mg/kg/day for seven days in drinking water and 10 control rats received no Indocin. All rats were monitored daily for weight and water consumption. Urine samples were obtained on day 5, 6, and 7. All rats were also observed daily for general appearance and activity. One rat receiving Indocin died on day 5 and a second Indocin rat died on day 6. Autopsies on these rats showed perforation of the small intestine. On day 7, all rats received 1150cGy abdominal radiation. One Indocin rat died from anesthesia, leaving a total of 7 Indocin rats and 10 control rats.

4.2.3 Results

The studies with intraperitoneal indomethacin showed no significant improvement in survival for any of the doses of abdominal radiation given (see table 8). At 1000 cGy, only one control rat died and no indomethacin rats died. At 1100cGy 10 percent of the controls and 30 percent of the indomethacin rats died, while 1200 cGy resulted in 30 and 40 percent mortality for the controls and indomethacin rats respectively. The highest dose of abdominal radiation used in this study, 1340cGy, resulted in the death of all 10 controls and 10 indomethacin rats by day 8.

Table 8. Percent mortality of rats receiving 4mg/kg indomethacin one hour prior to a dose of 1000, 1100, 1200 or 1340cGy abdominal radiation from the General Electric Maximar.

Radiation Dose:	1000		1100		1200		1340	
Days After Radiation (n)	Cont (10)	Ind (10)	Cont (10)	Ind (10)	Cont (9)	Ind (10)	Cont (10)	Ind (10)
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	10	10	11	10	10	10
5	10	0	10	30	11	10	30	50
6	10	0	10	30	33	20	70	60
7	10	0	10	30	44	30	100	70
8	10	0	10	30	44	30	100	100
9	10	0	10	30	44	20		
10	10	0	10	30	44	30		
11	10	0	10	30	44	30		
12	10	0	10	30	44	30		
13	10	0	10	30	44	30		
14	10	0	10	30	44	30		

Rats given 4mg/kg indomethacin prior to abdominal radiation, and receiving 1mg/kg/day for seven days by minipump also showed no decrease in mortality when compared to controls. (see table 9). However, it should be noted that none of the radiated control rats died compared to previous mortality rates of 80 to 100% following 1100cGy abdominal radiation from the linear accelerator. Because of the lack of deaths, no conclusions could be drawn. This may have related to employment of a smaller field.

Table 9. Percent mortality of rats receiving 4mg/kg indomethacin one hour prior to 1100cGy abdominal radiation from the linear accelerator and 1mg/kg/day by minipump for 7 days following radiation.

Day After Radiation (n)	Control (10)	Indocin (10)
1	0	0
2	0	0
3	0	0
4	0	0
5	0	10
6	0	20
7	0	20
8	0	20
9	0	20
10	0	20
11	0	20
12	0	20
13	0	20
14	0	20

Results of the toxicity studies of oral Indocin are given in table 10. Rats receiving Indocin in drinking water consumed more water and therefore more Indocin than the calculated dose, while rats receiving Indocin in food tended to consume less food and therefore less Indocin than the calculated dose. All water Indocin rats showed a weight loss during the 6 day period while four of the food Indocin rats showed the same or increased weight by day 6. Percent water content of the small intestine at the time of sacrifice showed somewhat higher values for the food Indocin rats than the water Indocin rats at all doses of Indocin used, but no significant difference from control values.

Table 10. Mean values of percent weight loss and percent water content for rats receiving Indocin food or Indocin water.

Intended Indocin Dose mg/kg/day	Number of Rats	Actual Dose mg/kg/day	%Wt Loss	%Water Content
5.0 water	3	8.0	3.3	75.7
10.0 water	3	15.7	5.8	72.0
20.0 water	3	29.0	4.1	75.2
5.0 food	3	4.3	4.3	76.2
10.0 food	3	8.0	+0.4	76.7
20.0 food	3	17.0	0.7	75.4
CONTROL	4	- -	- -	75.0

24-hour urinary Prostaglandin E excretion decreased for all animals receiving oral indomethacin for six days regardless of dose of indomethacin (table 11). Rats receiving water-indomethacin showed a greater decrease in prostaglandin E excretion than rats receiving food Indocin, as expected, because these rats received higher actual doses of indomethacin due to greater consumption of water. There did not appear to be any direct correlation between indomethacin dose and the amount of decreased prostaglandin excretion, but that could be due to the small size of the groups (n=2). The decreased excretion of PGE in the urine reflects the decreased prostaglandin synthesis caused by indomethacin.

Table 11. 24-hour urinary Prostaglandin E excretion for rats receiving oral indomethacin.

Indocin Dose mg/kg/day	Mean ng/24 Hr PGE Day 0 (n)	Mean ng/24 Hr PGE Day 6 (n)
5 water	83.5 (2)	58.5 (2)
10 water	125.5 (2)	68.5 (2)
20 water	104.0 (2)	77.0 (2)
5 food	100.5 (2)	85.5 (2)
10 food	80.5 (2)	78.0 (2)
20 food	119.0 (1)	92.0 (1)

Histological evaluation of tissues from rats receiving oral indomethacin is summarized in table 12. When compared to non-simultaneous control tissues, all tissues appeared to show a trend towards decrease in mucosal height regardless of the dose of indomethacin given.

Table 12. Mean mucosal height of small intestine for control rats and rats receiving oral indomethacin.

		S M A L L	I N T E S T I N E	
mg/k/day Indocin	(n)	Mean mm Ht. Proximal(SEM)	Mean mm Ht Mid (SEM)	Mean mm Ht. Distal (SEM)
5 water	3	0.507	0.508	0.498
10 water	3	0.448	0.473	0.422
20 water	3	0.493	0.510	0.424
5 food	3	0.478	0.473	0.440
10 food	3	0.543	0.473	0.491
20 food	3	0.477	0.429	0.470
Control	5	0.584 (.030)	0.582 (.018)	0.549 (.012)

Based on the previous study, it was determined to use a dose of 20mg/kg/day in the drinking water for seven days prior to 1150cGy abdominal radiation from the Maximar machine. The mortality data are given in table 13. There was no improvement in survival for rats receiving 20mg/kg/day oral Indocin for seven days prior to 1150cGy abdominal radiation.

Table 13. Percent mortality of control rats and rats receiving 20mg/kg/day Indocin water for seven days and 1150cGy abdominal radiation.

Days After Radiation	Control	Indocin
1	0	0
2	0	14
3	0	14
4	60	57
5	90	86
6	90	86
7	90	86
8	90	100

An early observation in our laboratory was that a single dose of indomethacin, administered parenterally one hour before an LD_{80} dose of abdominal radiation, provided significant protection against death. We repeated this experiment with variations in dosage of radiation delivered. The results, subsequent to the initial experiments, have been ambiguous but did not support the protection that was originally observed. The explanation for this discrepancy is not clear. Table 14 summarizes early versus later experiments.

Table 14. Mortality after abdominal radiation.

14a. 1100cGy Linear Accelerator, 4MeV "Early Experiments"

Days After Radiation	PERCENT MORTALITY n=25 Indocin	n=25 Control
1	0	0
2	0	0
3	0	0
4	13	40
5	13	52
6	13	52
7	20	52
8	20	52
9	20	60
10	20	60
11	20	60
12	20	60
13	20	64
14	20	64

14b. 220 KVp x-ray "Late Experiments"

	PERCENT MORTALITY					
Days	1100cGy		1200cGy		1340cGy	
After	n=10	n=10	n=10	n=9	n=10	n=10
Radiation	Indocin	Control	Indocin	Control	Indocin	Control
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	10
4	10	10	10	0	0	30
5	30	10	10	11	50	70
6	30	10	20	33	60	100
7	30	10	30	44	70	100
8	30	10	30	44	100	100
9	30	10	30	44	100	100
10	30	10	30	44	100	100
11	30	10	30	44	100	100
12	30	10	30	44	100	100
13	30	10	30	44	100	100
14	30	10	30	44	100	100

4.2.4 Discussion

Our initial observations were that indomethacin pretreatment provided improved survival after abdominal radiation. These results were the major impetus to develop the hypothesis regarding the role of prostaglandins in acute radiation enteritis.

The subsequent studies, described here, failed to duplicate the initial results. Systemic indomethacin given in various doses just before abdominal radiation did not prove to enhance survival. Neither did chronic administration by mouth alter the post radiation mortality curve. It is of importance to the overall issue of the role of prostaglandins that Indocin blockade did not *enhance* mortality. If, as some investigators have proposed, prostaglandins are radioprotective, then we might have anticipated a deleterious influence of prostaglandin inhibition. Such was not observed.

4.3 ASPIRIN

4.3.1 Background

Aspirin is one of the most commonly used of the NOSAC compounds and had been employed clinically to alleviate radiation sickness.[23] This group of agents blocks the activity of cyclooxygenase and thus inhibits the generation of prostaglandins.[25]

4.3.2 Methods

Lysine acetyl salicylate (Lorex Pharmaceuticals, Skokie, IL) is an injectable form of aspirin, and was used for these experiments. 250mg/kg was given intraperitoneally one hour before an abdominal radiation dose of 1000, 1100, 1350, 1500, or 1640cGy, using the smaller field.

4.3.3 Results

The survival results of rats given 250mg/kg IP lysine acetyl salicylate one hour prior to an abdominal radiation dose of 1000, 1100, 1350, 1500, or 1640cGy are given in table 15. At the dosage levels and route used, aspirin did not alter the mortality curves following abdominal radiation.

Table 15. Percent mortality following 1000, 1100, 1340, 1500, or 1640cGy abdominal radiation given 1 hour after 250mg/kg intraperitoneal lysine acetyl salicylate.

Radiation Dose:	1000		1100		1340		1500		1640	
Day After Radiation (n)	Cont (10)	ASA (10)	Cont (10)	ASA (9)	Cont (10)	ASA (10)	Cont (10)	ASA (10)	Cont (10)	ASA (10)
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	10	0	10	10	30	20	10	20
5	10	0	10	11	30	80	100	100	100	90
6	10	0	10	11	70	100	100	100	100	100
7	10	0	10	11	100	100				
8	10	0	10	11						
9	10	0	10	11						
10	10	0	10	11						
11	10	0	10	11						
12	10	0	10	11						
13	10	0	10	11						
14	10	0	10	11						

4.4 IBUPROFEN

4.4.1 Background

Ibuprofen is another non-steroid anti-inflammatory agent which has action similar to that of indomethacin, cyclooxygenase inhibition.

4.4.2 Methods

10mg/kg intraperitoneal ibuprofen, approximately one half the LD_{50}, was given an hour prior to 1150 or 1500cGy abdominal radiation in exactly the same protocol as described above.

4.4.3 Results

Intraperitoneal ibuprofen 10mg/k did not alter the mortality of rats subjected to 1150 or 1500cGy abdominal radiation when compared to control animals (table 16).

Table 16. Percent mortality of rats receiving 10mg/kg ibuprofen intraperitoneally one hour prior to 1150 or 1500cGy abdominal radiation.

Radiation Dose:	1150cGy		1500cGy	
Day After Radiation (n)	Cont (17)	Ibu (18)	Cont (10)	Ibu (9)
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	11	10	22
5	35	50	90	89
6	47	61	90	89
7	47	67	90	89
8	47	67	90	89
9	47	67	90	89
10	47	67	90	89
11	47	67	90	89
12	47	67	90	89
13	47	67	90	89
14	47	67	90	89

4.5 BENOXAPROFEN

4.5.1 Background

The enzyme lipoxygenase induces oxygenation of arachidonic acid which proceeds in a series of steps through HPETE to a group of compounds termed leukotrienes. One particularly potent member of this family, LTB_4, is chemotactic and causes leukocyte adhesion to vascular endothelium. Other leukotrienes enhance vascular permeability.[5,12,35] The vasodilator properties of prostaglandins PGE_2 and PGI_2 (prostacyclin) probably are synergistic with the leukotrienes in stimulating edema formation. It should be recalled that major effects of radiation injury to the intestine are vasodilation, interstitial edema and inflammatory cell infiltration, all of which can be caused by leukotrienes plus prostaglandins.

Benoxaprofen is a potent anti-inflammatory agent which has a relatively weak effect as a cyclooxygenase inhibitor and therefore on prostaglandin synthesis. Its major action is to block the lipoxygenase pathway.[5,12] Its effects on radiation injury could be fundamentally different from those of indomethacin.

4.5.2 Methods

Because benoxaprofen is only slightly soluble in water, dimethylsulfoxide (DMSO) was used as the solvent. Rats were given 125mg/kg prior to 1100cGy abdominal radiation in exactly the same protocol as indomethacin. The LD_{50} of benoxaprofen in the rat is 250mg/kg as reported by the manufacturer.

4.5.3 Results

There was no difference in survival in rats receiving 125mg/kg IP benoxaprofen one hour prior to 1100cGy abdominal radiation when compared to control rats receiving no drug (see table 17).

Table 17. Percent mortality for control rats and rats receiving 125mg/kg intraperitoneal benoxaprofen one hour prior to 1100cGy abdominal radiation.

Day After Radiation	(n=10) Control	(n=10) Benoxaprofen
1	0	0
2	0	0
3	0	0
4	10	0
5	40	50
6	50	60
7	80	70
8	80	70
9	80	70
10	80	70
11	80	70
12	80	70
13	80	70
14	80	70

4.6 QUINACRINE

4.6.1 Background

Phospholipase A is an enzyme responsible for release of arachidonic acid from the cell membrane. This enzymatic cleavage of arachidonic acid from precursor phospholipid is believed to be the rate limiting step in prostaglandin synthesis. Quinacrine hydrochloride is a phospholipase A_2 inhibitor.[38] Thomas and Knoop have shown that quinacrine pretreatment caused significant reduction in intestinal fluid outpouring (enteropooling) after topical mucosal exposure to heat stable *E. coli* enterotoxin.[41] It was postulated that this effect was dependent on blockade of phospholipase A, inhibiting transformation of membrane triglycerides to arachidonic acid, diminishing prostaglandin synthesis and also denying substrate to the lipoxygenase pathway. The investigators concluded that prostaglandins play an important role in the intestinal response to *E. coli* enterotoxin.

4.6.2 Methods

Toxicity studies for quinacrine showed that at a dose of 50mg/kg the rat displayed slow movements but resumed normal activity 24 hours later. At a dose of 100mg/kg, rats died within 10 minutes of the IP injection. Giving IP ketamine one hour after quinacrine administration resulted in both the 50 and 100mg/kg rats dying within 15 minutes of the ketamine. Rats given 25mg/kg quinacrine plus ketamine survived.

Quinacrine hydrochloride was dissolved in potassium phosphate buffer and administered 25mg/k by intraperitoneal injection one hour prior to 1150 or 1500cGy abdominal radiation. The protocol was otherwise the same as described above.

4.6.3 Results

The results of quinacrine hydrochloride, 25mg/kg given intraperitoneally one hour prior to 1150 or 1500cGy abdominal radiation are given in table 18. On post-radiation day 6, there was a significant difference in survival of animals given quinacrine pretreatment compared to control rats for both 1150 and 1500cGy. ($P = 0.013$) It appears that quinacrine hydrochloride in a dose of 25mg/kg given one hour prior to abdominal radiation may provide modest protection from intestinal death.

Table 18. Percent mortality for control rats and rats given 25mg/kg intraperitoneal quinacrine hydrochloride one hour prior to 1150 or 1500cGy abdominal radiation.

Radiation Dose:	Tube 3 1150cGy		Tube 2 1500cGy		Tube 1 1500cGy	
Day After Radiation	Large field		Small field		Small field	
(n)	Cont (10)	Quin (10)	Cont (10)	Quin (10)	Cont (10)	Quin (9)
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	30	30	30	0	10	0
6	80	50	60	10	30	22
7	80	60	80	30	30	33
8	80	60	100	50	40	33
9	80	60	100	80	40	33
10	80	60	100	90	50	33
11	80	60	100	90	60	33
12	80	60	100	90	70	33
13	80	60	100	90	80	33
14	80	60	100	90		

4.7 DIETARY DEPLETION OF PROSTAGLANDINS

4.7.1 Background

Diet has been shown to be a factor in intestinal sensitivity to radiation.[9] Cox, et al from Ferris' laboratory at the University of Minnesota have developed and published a method for inducing essential fatty acid deficiency in adult rats by total fasting followed by an essential fatty acid deficient diet(FAD).[7] In these animals urinary prostaglandin excretion fell to 20% of normal. Cox pointed out that experiments in which cyclooxygenase inhibitors were used often showed conflicting results because each agent has diverse effects, some of which are independent of prostaglandin inhibition. This dietary model for prostaglandin deficiency avoids the potential ambiguities inherent in the use of drugs which have multiple and complex actions.

For a pilot experiment we obtained three prostaglandin depleted rats from this laboratory. The rats were subjected to 1100cGy abdominal radiation. All three animals survived for 60 days at which time they were sacrificed. Without drug treatment, all three would have been expected to die from the intestinal radiation syndrome. This observation encouraged us to pursue further experiments with the dietary depletion model.

4.7.2 Methods

Male Sprague-Dawley rats from Harlan, Inc., Madison, WI. weighing 300-325 grams were used in this study. The dietary protocol used was developed by Cox et al. Rats were fasted until a 25% weight loss was attained, generally seven days, but were allowed free access to water. They were then given 10% dextrose in drinking water for the next four days. The rats were weighed daily during the fasting and dextrose periods.

At the end of the fast they were divided into four groups. Group number one received a diet deficient in essential fatty acids (Teklad #79131, Madison, WI.) for 15 days. Of the 25 rats in this group, 15 were followed for survival and 10 were sacrificed sequentially, two each on days four through eight post radiation for histologic study of the intestine.

Group two, 5 rats, received the deficient diet but were never subjected to radiation. After 15 days on the deficient diet the rats showed some skin scaling and slight hair loss but otherwise appeared healthy and active and had regained their prefasting weight.

The rats in group number three received a normal control diet (#79132, Teklad Laboratories, Madison, WI) for fifteen days after fasting and dextrose water preparation, then were subjected to abdominal radiation. Fifteen of these rats were followed for survival and 10 were sacrificed sequentially, two per day on days four through eight, for histologic evaluation of the intestine.

The 5 rats in group number four received the control diet but no radiation. Abdominal radiation was given as described above. After radiation animals were allowed to recover from anesthetic and were returned to cages to continue the specified diet and water ad lib.

A second experiment was done using a 4MeV Linear Accelerator instead of x-irradiation from the Maximar machine. Ten rats were prepared with the FAD diet and ten with the control diet as described above. Shielding of the limbs and thorax was provided by lead collimators. The area irradiated extended from xiphoid to pubis. A single dose of 1100cGy was delivered by the 4 MeV Linear Accelerator. The calculated dose rate, at the abdomen of each animal, was 320cGy/minute. The source-to-skin distance was 80 centimeters.

Rats followed for survival were observed every post-radiation day and all deaths recorded. The final groups in the first study included thirteen rats receiving the control diet plus radiation, and fifteen rats receiving the FAD diet plus radiation.

Urinary prostaglandins, assayed according to the method of Venuto, et al,[43] total leucocyte counts, and intestinal mucosa for histologic injury quantitation were taken from these animals.

4.7.3 Results

Following X radiation, fatty acid deficient rats showed increased survival compared to the rats receiving the control diet. Using the Chi-Square test, there was a significant difference in survival on post radiation days six, seven, eight, and nine. ($P<0.05$). See figure 3.

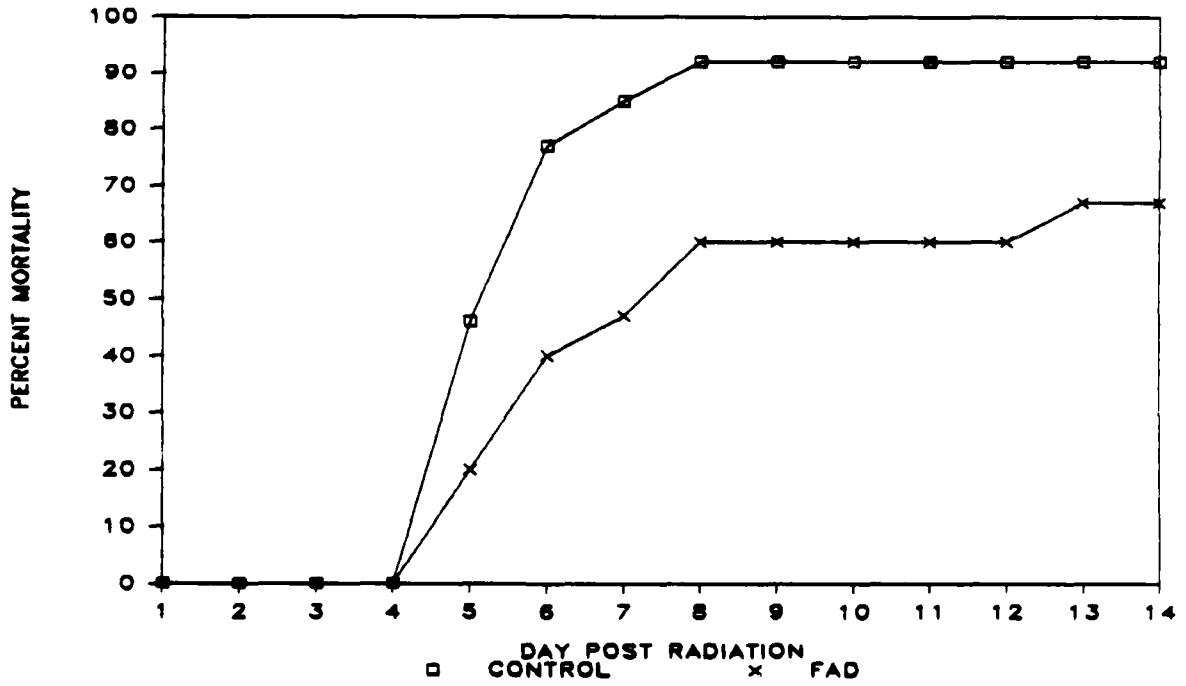


Figure 3. Percent mortality for FAD diet rats compared to control diet rats following 1400cGy abdominal X radiation.

Animals receiving 1100cGy from the Linear Accelerator also showed better survival for rats on the FAD diet compared to those on the control diet. However, these numbers failed to reach statistical significance using the Chi-Square test. See figure 4.

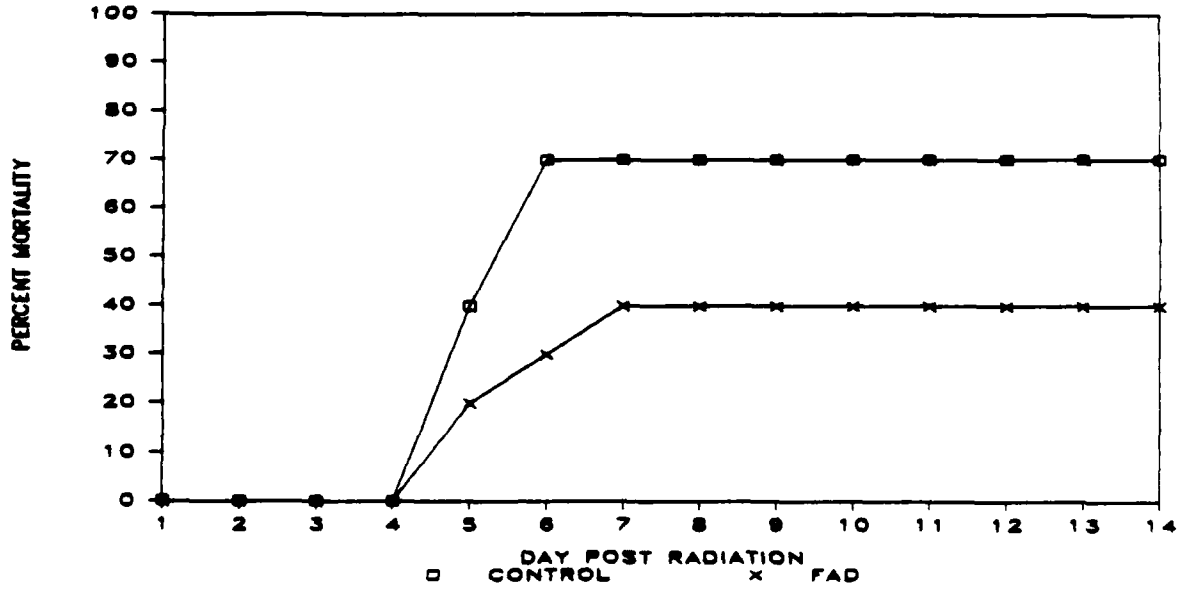


Figure 4. Percent mortality for FAD diet rats and control diet rats following 1100cGy linear accelerator abdominal radiation.

Urinary prostaglandin excretion was determined for the FAD diet rats and for the regular diet rats according to the methods described under Urinary Prostaglandin Excretion Following Abdominal or Intestinal Radiation, page 41. After the fast regimen and 13 days on the deficient diet, mean urinary PGE excretion was 25.29ng/24 hours (SEM=2.3) for 24 samples from 8 rats. For corresponding control diet rats, mean 24 hour urinary PGE excretion was 38.71ng/24 hours (SEM=4.3) for 23 samples from 8 rats. All rats showed a decrease in urinary PGE excretion regardless of whether they were given the control diet or the deficient diet. This could possibly be a consequence of the fasting and weight loss protocol all rats go through prior to being given the designated diets. At the end of the diet period, rats on the control diet excreted approximately half the amount of PGE that they excreted before any treatment, while rats on the deficient diet excreted approximately one third the amount found prior to any treatment (see table 19).

Table 19. PGE excretion for control and fatty acid deficient rats in mean ng/24 hours.

Group	ng/24 hours Before Diet Treatment(SEM)	ng/24 hours After 15 days days on diet (SEM)
Control Diet	84.87 (6.9) n=18	38.71 (4.3) n=23
Deficient Diet	68.04 (3.8) n=21	25.29 (2.3) n=24
		P<0.01

Total leukocyte counts were determined in rats in each of the four groups prior to radiation and until day 6. These results are given in table 20. There was no difference in total leukocyte counts for the deficient diet rats compared to the control diet rats. There was also no difference between the radiated FAD rats and the radiated control diet rats.

Table 20. Mean total leukocyte counts for fatty acid deficient and for control rats.

Group	Before Radiation $x10^3$/ul	Day 2 $x10^3$/ul	Day 4 $x10^3$/ul	Day 6 $x10^3$/ul
FAD diet, +no rad	10.9 (0.9)	10.7 (0.5)	9.1 (0.7)	9.3 (0.8)
n:	5	5	5	5
FAD diet,+1400cGy	9.7 (0.3)	3.6 (0.1)	3.7 (.07)	6.3 (0.6)
n:	25	25	25	13
Control, +no rad	10.5 (0.7)	10.3 (0.5)	11.0 (0.6)	9.9 (0.7)
n:	5	5	5	5
Control, +1400cGy	10.5 (0.4)	2.5 (.09)	5.1 (0.3)	4.4 (0.6)
n:	23	22	23	8

Histological evaluation showed radiated FAD rats were no different in mucosal height from rats that received the control diet plus radiation. See table 21.

Table 21. Mean mucosa height for fatty acid deficient diet and control diet rats with no radiation and for deficient diet and control diet plus 1400cGy Maximar abdominal radiation.

	mm H E I G H T M U C O S A				
Group	Day 4(n)	Day 5(n)	Day 6(n)	Day 7(n)	Day 8(n)
FAD diet, +no rad	0.672(2)	0.705(2)	0.699(2)	0.689(2)	0.754(2)
Control, +no rad	0.628(2)	0.718(2)	0.665(2)	0.677(2)	0.616(2)
FAD diet, +1400cGy	0.336(2)	0.253(2)	0.271(1)	0.437(1)	0.365(1)
Control, +1400cGy	0.284(2)	- - -	0.236(2)	0.443(2)	0.358(2)

4.8 PROSTAGLANDIN INHIBITION AND HEMATOPOIESIS FOLLOWING ABDOMINAL RADIATION

4.8.1 Background

Peripheral circulatory leukocyte numbers decrease in a predictable pattern following radiation.[6] In survivors of radiation injury the peripheral leukocyte count recovers with a similarly predictable time course. If leukocytes fail to reappear in the circulation, the animal dies a late "hematopoietic" death from infection. This course depends on species and radiation parameters. These studies addressed the question of whether prostaglandin inhibition could protect hematopoietic function.

4.8.2 Methods

Ten rats were subjected to 500cGy *whole body* radiation. Five had received 4mg/kg intraperitoneal Indocin one hour prior to radiation. Blood samples were obtained by tail vein puncture. Total leukocyte counts were measured on a Coulter counter before radiation and on post-radiation days 1, 3, 5, 7, 14, and 21. Leukocyte counts were also obtained for two groups of rats that received 1100cGy abdominal radiation from the linear accelerator: rats that received 125mg/kg intraperitoneal benoxaprofen one hour prior to radiation (p.29) and rats subjected to the dietary depletion of prostaglandins protocol (p.32-33).

4.8.3 Results

Leukocyte counts for the control animals receiving 500cGy whole body radiation fell to a low of 1.5 on post-radiation day 5, and then rose progressively to 9.4 on day 27. The Indocin pre-treated animals showed a low WBC of 1.3 on day 5 and 8.8 on day 27. While these differences are not significant, WBC for the indocin treated rats showed a faster recovery on days 7, 14, and 21 (see table 22).

Table 22. Mean total peripheral leukocyte counts for rats prior to linear accelerator radiation.

Radiation:	500cGy Whole Body		1100 cGy Abdominal		
	Control	Indocin	Benoxaprofen	FAD	Control
Day (n)	(5)	(5)	(n)	(n)	(n)
0	9.7	9.2	11.7 (10)	9.6 (10)	11.1 (10)
1	4.1	5.4			
3	2.4	4.1	2.1 (10)	3.8 (10)	3.8 (10)
5	1.5	1.3	2.2 (5)		
6				4.9 (7)	3.1 (3)
7	1.6	2.1	2.3 (3)		
10			9.0 (3)	7.0 (6)	4.5 (3)
12			11.6 (3)	8.5 (6)	8.3 (3)
14	2.7	4.4			
21	4.0	5.8		10.4 (6)	10.2 (3)
28	9.4	8.8	10.8 (3)	5.0 (6)	4.5 (3)

Rats receiving benoxaprofen one hour prior to 1100cGy linear accelerator abdominal radiation showed their lowest mean leukocyte count on day 3. By day 10, the mean leukocyte count for surviving rats had returned to pre-radiation level. Fatty acid deficient (FAD) rats reached their lowest mean value of 3.8 on day 3 after which time leukocyte counts began to rise reaching beginning values between day 12 and 21. Corresponding control rats had their lowest counts of 3.1 on day 6 and also returned to normal values between day 14 and 21. These differences were not significant. Rats receiving whole body radiation took a longer period of time for leukocyte counts to return to control levels (between 21 and 27 days) compared to rats receiving abdominal radiation.

From these various observations, we concluded that prostaglandin manipulations did not alter leucocyte depression or recovery following whole body or abdominal radiation.

4.9 ENTEROPOOLING

4.9.1 Background

One of the major features of the intestinal syndrome following radiation injury is diarrhea. As noted previously, prostaglandins can by themselves induce diarrhea. Intraluminal cholera toxin or heat stable *E. coli* enterotoxin both cause excess fluid secretion into the intestinal lumen and consequent diarrhea.[17] Both agents also induce elevated levels of prostaglandin E_2 in the intestinal wall.[18,41] Furthermore, intestinal fluid response to these agents can be blocked by indomethacin inhibition of prostaglandin production.[14,36] If prostaglandin generation plays an important role in radiation injury to the intestine, radiation would be expected to induce fluid outpouring into the lumen which should be blocked by cyclooxygenase inhibitors.

The phenomenon of fluid accumulation in the small intestine is called "enteropooling".[34] The experimental method of grading the relative diarrheagenic activity of prostaglandins has been termed "the enteropooling assay". The volume of fluid in the lumen is the sum of fluid secreted plus the fluid already present whose absorption is inhibited by prostaglandins.[45]

4.9.2 Methods

Rats were fasted overnight to reduce the food content of the small intestine. The animals were kept in special cages to prevent coprophagy. Four treatment groups were included in this study. The first group was control animals that received no drug and no radiation. The second group received anesthesia and an intraperitoneal injection of saline. The third group received anesthesia and 0.5mg/k oral 16,16-dimethyl PGE_2 one hour prior to sacrifice. The fourth group received anesthesia and 1500cGy abdominal radiation.

Following experimental treatment rats from the anesthesia and abdominal radiation group were sacrificed at 60, 120, and 240 minutes, while all of the rats in the control group and in the PGE_2 group were sacrificed at one hour. The pylorus and the ileocecal junction were tied and the intestine, with its contents, was dissected free of surrounding tissues and weighed. The contents were milked out and collected in a graduated tube to measure volume. The intestine was then reweighed, desiccated for 48 hours to dryness and then weighed again. The wet weights and the dry weights of the intestine were used to quantify edema within the intestinal wall.

4.9.3 Results

The fluid content of the intestinal lumen following prostaglandin administration was significantly increased over control. However, one, two and four hours following radiation, we were unable to discern any increase in the intestinal wall fluid content over control. Table 23 gives the fluid content of the intestinal lumen and table 24 gives the mean percent water of the wall of the small intestine.

Table 23. Mean volume of fluid in small intestine 1, 2 and 4 hours after anesthesia, 16,16-dimethyl PGE_2, or 1500cGy abdominal radiation.

Treatment	ml Volume	SEM
Control	1.048	0.22
16,16-dimethyl PGE_2 + 1 hour	*2.974	0.79
1500cGy + 1 hour	1.662	0.50
1500cGy + 2 hour	1.360	0.22
1500cGy + 4 hour	1.271	0.07

*P=.042 compared to control

Table 24. Percent water in small intestinal wall 1, 2, and 4 hours after anesthesia, 16,16-dimethyl PGE_2, or 1500cGy abdominal radiation.

GROUP	HOURS	% WATER
Control	1	75
Anesthesia	1	78
	2	75
	4	78
16,16-PGE_2	1	74
1500cGy Radiation	1	73
	2	75
	4	75

We conclude that radiation does not induce enteropooling or early mural edema while oral prostaglandin does cause enteropooling.

4.10 URINARY PROSTAGLANDIN EXCRETION FOLLOWING ABDOMINAL OR INTESTINAL RADIATION

4.10.1 Background

We have harvested numerous tissue samples following radiation to assay prostaglandin content. Many studies have been done which measure prostaglandin content in tissues.[19,39,40] However, in reviewing the most recent literature, it appears that the present consensus view is that measured prostaglandin content of tissue is more a function of harvesting technique than it is of physiologic levels. We therefore, have confined the prostaglandin assays to 24-hour urine samples.

4.10.2 Methods

Rats were housed individually in metabolic cages for 24 hour urine sample collection. The total volume of urine was measured and the sample was frozen until later assayed for prostaglandin E (PGE) and/or 6-keto-prostaglandin F_1 alpha (6-keto-PGF_{1a}) by radioimmunoassay.[4,43]

The antibodies for the assay were raised in New Zealand rabbits by repeated injections of PGE_2 or 6-keto-PGF_{1a} linked to thyroglobulin and suspended in Freund's adjuvant. Cross reactivities for the PGE antibody were PGE, 40%; 6-keto-PGF_{1a}, 2%; PGB_2, 1%; PGA_2, 3%; PGF_{1a}, 1%. Results of the radioimmunoassay are expressed as urinary PGE because there is significant cross reactivity with PGE1. Cross reactivities for the 6-keto-PGF_{1a} antibody were: PGE, 2%; PGF_{1a}, 1.5%; PGE_2, PGA_2, and PGB_2 < 1%. Urinary PGE was measured according to the method of Zusman, [46] as modified by Vento, et al.[43] Samples were acidified with citric acid to a pH of 3.7. They were then extracted with ethyl acetate, dried under air, and applied to a silicic acid column. [^{3}H]PGE_2 (New England Nuclear) was added to each tube, and recovery was 62 +/- 3%. Coefficients of variation were 10% intraassay and 20% interassay. The assay detected PGE above 50pg/ml.

Diluted unextracted urine was used for the measurement of 6-keto-PGF_{1a}. The intraassay coefficient of variation was 3% and the interassay coefficient of variation was 4%. The lower limit of detectability was 25 pg/ml.

To measure control urinary prostaglandins, three 24-hour urine sample were obtained as described above from five rats prior to 1100cGy linear accelerator abdominal radiation. These rats received no drug injection of any kind. Twenty-four hour urine samples were then obtained for post-radiation days 1 through 3.

In a second study, 24-hour urine samples were obtained from a group of three rats for three days prior to 1100cGy Maximar radiation to 70% of the exteriorized small intestine, and for the first four days following radiation. The animals were anesthetized and shielded in lead boxes. The intestine was exteriorized via laparotomy and pulled through a hole in the lead box to be radiated, essentially as described on page 59. These samples were assayed for PGE and for 6-keto-Prostaglandin F_{1a}.

Urinary prostaglandin levels were also determined for rats in the Fatty Acid Deficient Diet experiment (see page 32). 24-hour urine samples were obtained from 8 rats receiving the deficient diet, and 8 rats on the normal diet for three days prior to beginning the dietary regimen and for the last three days prior to radiation, days 13, 15, and 15 of the designated diet. This choice of sampling time allowed us to look at the effect of the fatty acid deficient diet by itself on urinary prostaglandin levels. These results are given on page 34.

4.10.3 Results

The mean for all post-radiation samples (n=15) was 37.29 +/-7.5, significantly reduced from the mean urinary PGE of 71.45ng/24 hours (SEM 7.4) prior to radiation ($P<0.002$). The individual values are given in table 25. The mean values for post-abdominal radiation days 1, 2 and 3 show a continued decrease in PGE.

Table 25. Urinary PGE excretion in nanograms(ng)/24 hours before and after abdominal radiation.

Rat #	1	2	3	4	5	DAILY MEAN (SEM)
Pre-Radiation Day						
3	78.20	--	83.72	38.42	46.50	61.71 (11.3)
2	70.21	65.94	58.32	32.64	52.80	55.98 (6.6)
1	115.75	87.36	104.00	117.99	48.50	94.72 (12.8)
					MEAN(n=14)	71.45 (7.4)
Post-Radiation Day						
1	59.50	25.83	43.42	29.50	123.20	56.29 (17.7)
2	29.50	30.00	43.20	22.50	66.22	38.28 (7.7)
3	13.52	32.00	4.00	14.40	22.92	17.37 (4.7)
					MEAN(n=15)	37.29 (7.5)

Results following radiation of the isolated intestine (table 26) showed an increase in PGE excretion for the first 24 hours following radiation, and successive decreases on post radiation days 2, 3, and 4. The mean for all pre-radiation samples was 81.46ng/24 hours (11.1). The mean for post-radiation day one was significantly increased (119.91+/-6.0) compared to control values, and the mean for post-radiation day 3 (57.79 +/-4.7) was significantly decreased compared to pre-radiation values. The values for post-radiation days 2 and 4 were not significantly different from the pre-radiation values.

The assay for 6-keto-PGF_{1a} gave a pre-radiation value of 35.52ng/24 hours. Post-radiation values were significantly increased (48.10ng/24 hours, P=0.042) on day 1 and near normal on days 2 (32.69), 3(23.49), and 4(30.53).

Table 26. Urinary PGE and 6-keto-PGF_{1a} before and after 1100cGy Maximar radiation to 70% of the exteriorized small intestine.

Pre-Radiation Day	PROSTAGLANDIN E Rat 1	2	3	MEAN (SEM)	6-KETO-PGF_{1a} 1	2	3	MEAN (SEM)
3	124.60	48.55	69.30	80.82 (22.7)	50.20	23.65	35.64	36.50 (7.7)
2	59.95	- -	82.08	71.02 (11.1)	30.58	- -	38.38	34.48 (3.2)
1	128.51	89.76	48.93	89.07 (23.0)	35.28	37.56	29.13	33.99 (2.5)
			MEAN(n=8)	81.46 (11.1)			MEAN(n=8)	35.52 (2.8)
Post Radiation Day								
1	112.60	115.29	131.84	119.91 (6.0)	46.35	52.20	45.76	48.10 (2.1)
2	91.50	63.14	79.70	78.11 (8.2)	36.26	29.12	32.70	32.69 (2.1)
3	49.00	58.80	64.98	57.79 (4.7)	24.12	22.60	23.76	23.49 (0.5)
4	105.50	27.50	45.75	59.58(23.6)	39.96	21.13	30.50	30.53 (5.4)
			MEAN(n=12)	78.80 (9.4)			MEAN(n=12)	33.71 (3.0)

These results are not readily interpreted. Intact abdominal radiation lead to reduced prostaglandin urinary excretion. Was this due to altered production or some deviation in renal clearance of prostaglandin? When the kidneys were shielded and only the intestine radiated, there was a consistent increase in urinary PGE excretion during the subsequent 24 hours followed by normal values the next three days. 6-Keto PGF_{1a} excretion showed the same pattern. One can speculate that the intestinal trauma inherent in exteriorization could have caused increased prostaglandin release unrelated to radiation injury. To pursue this question would require additional studies of the effects of exteriorization alone.

SECTION 5

ADDITIONAL SURVIVAL STUDIES

5.1 ALLOPURINOL

5.1.1 Background

Allopurinol is a xanthine oxidase inhibitor. The rationale for its use is that xanthine oxidase participates in mucosal generation of superoxide. Experimentally, allopurinol has proved protective against ischemia reperfusion injury of the intestine. Radiation damage is somewhat analogous with respect to free radical generation.[13]

5.1.2 Methods

Allopurinol, 250mg/kg in 0.5ml saline was injected intraperitoneally in ten rats one hour prior to 1400cGy abdominal radiation, field size of 4.0 x 5.5cm, from the General Electric Maximar machine. Ten control rats received 0.5ml IP one hour prior to radiation. In a second study, nine rats received 250mg/kg/day by gavage for two days. On the third day, these rats received 250mg/kg by tail vein IV immediately prior to 1500cGy abdominal radiation from the Maximar machine. 1500cGy was used for this study because 1400cGy in the immediately previous study resulted in only 50% mortality in the control rats.

5.1.3 Results

Percent mortality for rats receiving allopurinol either by intraperitoneal injection or by gavage and tail vein IV are given in table 27. Allopurinol given intraperitoneally one hour prior to abdominal radiation did not favorably alter mortality. Rats receiving 3 days of oral allopurinol followed by IV drug immediately prior to radiation had greater mortality than control rats, although not statistically significant.

Table 27. Percent mortality for rats receiving 250mg/kg allopurinol intraperitoneally or by gavage and tail vein prior to abdominal radiation.

	1400 cGy IP		1500 cGy Oral+IV	
Day After Radiation	Control (n=10)	Allopur. (n=10)	Control (n=9)	Allopur. (n=9)
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	10	10	11	11
6	20	10	11	56
7	20	10	22	78
8	30	20	44	78
9	30	30	44	89
10	30	40	44	89
11	50	40	67	100
12	50	50	67	100
13	50	50	78	100
14	50	60	78	100

5.2 BACITRACIN AND STREPTOMYCIN

5.2.1 Background

Lumenal bacteria may play a role in the intestinal syndrome. Germ-free animals have been shown to survive a lethal dose of whole body radiation for a slightly longer time than did a control group.[22] Bacitracin plus streptomycin given in the drinking water for four days "decontaminates" the gut, ie, reduces bacterial concentrations to a very low level. This study was designed to test the possibility that a greatly reduced lumenal bacterial burden might alter the intestinal syndrome following *abdominal* radiation.

5.2.2 Methods

Ten rats received 2mg bacitracin and 2mg streptomycin/ml tap water, mixed fresh daily, in the drinking water for four days prior to 1200cGy 4.5 x 8cm abdominal radiation from the Maximar machine, and continuing after radiation until the end of the study. A daily record of water consumption was kept to determine the actual intake of the drugs. The above experiment was repeated in 20 rats using a radiation dose of 1000cGy.

5.2.3 Results

Prior to radiation, the control rats consumed more water per day by a factor of 2 than did the rats receiving bacitracin and streptomycin in the water. The actual dose of bacitracin and streptomycin received prior to radiation was 47.6mg/rat/day, or 159mg/kg/day. With an abdominal radiation dose of 1200cGy, oral bacitracin and streptomycin did not alter the percent mortality when compared to corresponding control rats. At a radiation dose of 1000cGy, there was a significant decrease in percent mortality of drug rats when compared to corresponding control rats. (P=.046). Mortality results are given in table 28.

Table 28. Percent mortality for control rats and rats receiving 2mg bacitracin and 2mg streptomycin per ml drinking water for four days prior to and until death following abdominal radiation.

	1200 cGy		1000 cGy	
Day After Radiation	Control (n=10)	Bac.& Strep. (n=10)	Control (n=10)	Bac.&Strep. (n=19)
1	0	0	0	0
2	0	0	0	0
3	0	10	0	0
4	20	10	0	0
5	80	30	30	0
6	90	50	40	5
7	90	60	40	5
8	90	70	40	11
9	90	80	40	16
10	90	80	40	16
11	90	80	40	21
12	90	90	40	21
13	90	90	40	21
14	90	90	50	26

5.3 BETAMETHASONE

5.3.1 Background

Glucocorticoids are adrenocortical steroids that are readily absorbed from the gastrointestinal tract. They have a "membrane stabilizing" effect and inhibit lipase release of arachidonic acid, the first step in the prostaglandin cascade. Acute high dose steroid pretreatment should in theory diminish prostaglandin production at the time of the radiation injury. Celestone is a powerful anti-inflammatory agent which has less sodium- and water-retaining properties than naturally occurring glucocorticoids such as hydrocortisone and cortisone.

5.3.2 Methods

Injectable Celestone Phosphate, (betamethasone sodium phosphate) 3mg/ml was obtained from Schering Corporation, Kenilworth, N.J. Rats received 6mg/kg intraperitoneally one hour prior to 1500cGy small field (4.5 x 5.0) abdominal radiation, Maximar machine. In a second study, 6mg/kg Celestone was injected intraperitoneally one hour prior to 1150cGy from the Maximar machine to the large abdominal field (4.5 x 8.0cm).

5.3.3 Results

Results of the studies using Celestone Phosphate are given in table 29. There was a significant decrease in mortality for both the small field 1500cGy (P=.007) and for the large field 1150cGy (P=.056) X radiation.

Table 29. Percent mortality following 6mg/kg IP Celestone Phosphate and 1500cGy or 1150cGy abdominal radiation Maximar machine.

	Small Field 1500 cGy		Large Field 1150 cGy	
Day After Radiation	Celestone (n=10)	Control (n=9)	Celestone (n=19)	Control (n=20)
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	56	11	30
6	20	78	16	45
7	40	89	21	50
8	50	89	21	50
9	50	89	21	50
10	50	89	21	50
11	60	89	21	50
12	60	89	21	50
13	60	89	21	50
14	60	89	21	50

5.4 DEXAMETHASONE

5.4.1 Background

Decadron (dexamethasone sodium phosphate), a synthetic adrenocortical steroid, is a potent anti-inflammatory agent which lacks the sodium-retaining properties of cortisone.

5.4.2 Methods

Injectable Decadron was obtained from Merck Sharp & Dohme, West Point, PA. as a stock solution containing 24mg/ml. Ten rats received 48mg/kg Decadron intraperitoneally one hour prior to 1500cGy abdominal radiation from the Maximar machine. Corresponding control rats received 0.5ml intraperitoneal saline one hour prior to radiation and all rats were followed for survival.

5.4.2 Results

Decadron, 48mg/kg given one hour prior to abdominal radiation allowed a 70% mortality while corresponding control rats had 100% mortality. These results are given in table 30. Decadron did significantly decrease percent mortality (P=.035).

Table 30. Percent mortality for controls and rats receiving 48mg/kg Decadron IP one hour prior to 1500cGy abdominal radiation from Maximar machine.

Day After Radiation	Control (n=10)	Decadron (n=10)
1	0	0
2	0	0
3	0	0
4	0	0
5	30	20
6	60	30
7	80	30
8	100	30
9	100	30
10	100	30
11	100	60
12	100	70
13	100	70
14	100	70

5.5 CHOLESTYRAMINE

5.5.1 Background

Bile acids during normal digestion are secreted into the intestine. A major portion of the bile acids is absorbed from the intestinal tract and returned to the liver via the enterohepatic circulation. Questran resin adsorbs and combines with the bile acids in the intestine to form an insoluble complex which is excreted in the feces. This results in a partial removal of bile acids from the circulation by preventing their absorption. More significantly for the present study the bile salts are bound in an inert form at the time of the radiation. We have previously shown that bile in the lumen enhances radiation damage to the mucosa.

5.5.2 Methods

Questran (cholestyramine resin) was obtained from Mead Johnson Pharmaceuticals, Evansville, Indiana. One packet, containing 4 grams of anhydrous cholestyramine resin, was mixed in 200ml drinking water and given to 10 rats 24 hours prior to 1100cGy abdominal radiation from the Maximar machine. In this study, the rats did not appear to have consumed much of the water - less than 10ml/rat in 24 hours. The mortality results are shown in table 31.

In a second study, 450ml tap water was added to 300gm normal lab chow. After soaking for 3 hours, one package of liquid cholestyramine was mixed with the lab chow and this slurry was dried overnight. This lab chow/cholestyramine mixture was given to ten rats for 7 days prior to 1100cGy abdominal radiation from the Maximar machine. The food was weighed at the beginning and end of each 24-hour period. The rats consumed an average of 22gm of food/day which equaled an approximate daily intake of 300mg cholestyramine/rat. Corresponding control rats received the same food mixture but without cholestyramine. Percent mortality results are given in table 31. In neither study did oral cholestyramine consumption improve survival over control rats.

Table 31. Percent Mortality for control rats and rats receiving oral Questran for 7 days prior to 1100cGy abdominal radiation from the Maximar machine.

Day After Radiation	Water Questran (n=10)	Control (n=10)	Food Questran (n=9)	Control (n=10)
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	80	40	33	0
6	90	50	56	40
7	90	70	78	70
8	90	90	89	70
9	90	90	100	90
10	90	90	100	90
11	90	90	100	90
12	90	90	100	90
13	90	90	100	90
14	90	90	100	90

5.6 DAZMEGREL

5.6.1 Background

Dazmagrel (UK-38,485) is a potent inhibitor of human blood platelet microsomal thromboxane (TxA_2) synthetase. Perry, et al.[30] found serum thromboxane B_2 levels were reduced by 88% after intravenous administration in rabbits and by 90% after oral administration in dogs.[30] Dazmagrel was provided by Nathan Belcher, Pfizer Central Research, Groton, Connecticut.

5.6.2 Methods

Dazmagrel, 20mg was dissolved in 2ml 0.1N sodium hydroxide. The pH was then adjusted to 7.0 with 0.1N hydrochloric acid, and saline was added to a total volume of 10ml. This resulted in a concentration of 2mg Dazmagrel/ml. Ten rats received 6mg/kg intraperitoneally one hour prior to 1500cGy small field abdominal radiation from the Maximar machine. Control rats received the same volume (0.75ml) of the solute one hour prior to radiation.

5.6.3 Results

Table 32 gives the percent mortality for these rats. There was no difference in mortality for rats receiving Dazmagrel one hour prior to abdominal radiation when compared to control rats receiving only solvent.

Table 32. Percent mortality for control rats and rats receiving 6mg/kg Dazmagrel one hour prior to 1500cGy abdominal radiation from the Maximar machine.

Day After Radiation	Dazmagrel (n=10)	Control (n=10)
1	0	0
2	0	0
3	0	0
4	0	10
5	50	40
6	70	40
7	70	50
8	70	90
9	80	90
10	90	90
11	90	90
12	90	90
13	90	90
14	90	90

5.7 DESFERAL

5.7.1 Background

Desferal (deferoxamine mesylate) has the specific ability to chelate iron, forming a stable complex which prevents the iron from entering into further chemical reactions. Very little of the drug is absorbed when administered orally. The basis for the study is that iron containing enzymes are essential to the reactions which generate free radicals. Iron deprivation might diminish free radical damage caused by radiation.[37]

5.7.2 Methods

Desferal was obtained from Ciba Pharmaceutical Company, Summit, N.J.. It is reported by the manufacturer to have an LD_{50} in the rat of 329mg/kg intravenously. We administered 350, 700, and 1500mg/kg intraperitoneally to two rats at each dose. All animals died within 24 hours, but rats receiving 200mg/kg survived.

In our first study with Desferal, six rats received 500mg in 200ml drinking water daily for 8 days. Desferal was mixed fresh daily and any water remaining from the previous day was measured. The average daily oral consumption for the 8 days was 138mg/rat (SEM=8.8) or 460mg/kg. On day 8, 6 Desferal and 6 control rats received 1100cGy abdominal radiation from the linear accelerator.

In a second study, nine rats received 50mg/kg intravenously by Harvard pump beginning 10 minutes before 1150cGy abdominal radiation from the Maximar machine. The intravenous infusion continued during the time radiation was being administered and for 5 minutes after radiation. The total time to administer the 50mg/kg Desferal was 25 minutes. Control rats received the same volume of intravenous saline (2ml) over 25 minutes, and all rats were followed for survival. The idea was to maximize iron binding during the critical time when radiation was delivered.

5.7.3 Results

Results of the Desferal studies are given in table 33. Oral Desferal for 8 days prior to abdominal radiation was associated with 83% mortality compared to 50% mortality for the control rats. The small number of animals in this study (n=6) makes it difficult to reach any conclusions, but there was a hint of protection.

Results of intravenous Desferal pretreatment also showed no difference between the control and the drug treated rats.

Table 33. Percent mortality for control rats and rats receiving oral or intravenous Desferal prior to abdominal radiation.

Day After Radiation	1500cGy Linear Acc. Oral Desferal (n=6)	Control (n=6)	1150cGy Maximar IV Desferal (n=9)	Control (n=10)
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	20	11
5	50	33	80	89
6	83	50	90	89
7	83	50	90	89
8	83	50	90	89
9	83	50	90	89
10	83	50	90	89
11	83	50	90	89
12	83	50	90	89
13	83	50	90	89
14	83	50	90	89

5.8 DIMETHYL SULFOXIDE

5.8.1 Background

Dimethyl sulfoxide is a classic free radical scavenger and has been studied extensively for this property.[21]

5.8.2 Methods

DMSO was obtained from Sigma Chemical Company, St. Louis, MO. One ml of liquid contains approximately 1.10gm DMSO. For toxicity studies in our laboratory, 2 rats each were injected intraperitoneally with 0.5, 1, 2, 3, 4, or 5ml DMSO. All rats reacted immediately to the injection as though feeling pain and then becoming somnolent and depressed. At this point none of the rats reacted to being handled. Rats receiving 5ml (20,000mg/kg) died within 30 minutes and rats receiving 4ml (16,000mg/kg) died between 90 and 120 minutes. Of the two rats receiving 3ml(12,000 mg/kg), one died within two hours and the second died on day 3, and rats receiving 2ml (8,000mg/kg) also died on day 3. The two rats receiving 1ml (4,000mg/kg) survived indefinitely with no apparent problem after the initial depression,as did the two rats receiving 0.5ml (2,000 mg/kg). We considered that 4000mg/kg injected intraperitoneally was a maximum non-lethal dose.

Rats received 1ml DMSO by intraperitoneal injection one hour prior to 1340, 1490, or 1639cGy abdominal radiation from the Maximar machine. Ten control rats for each of the three doses of radiation received 1ml intraperitoneal saline one hour prior to radiation.

5.8.3 Results

Rats receiving 1ml DMSO one hour prior to abdominal radiation showed no improvement in mortality over control rats for abdominal radiation doses of 1340, 1490, or 1639cGy. Although the control rats appeared to die sooner, there was no significant difference in the percent mortality for any of the doses of radiation given (see table 34).

Table 34. Percent mortality for control rats and rats receiving 4,000mg/kg DMSO one hour prior to abdominal radiation from the Maximar machine.

	1340 cGy		1490 cGy		1639 cGy	
Day After Radiation	DMSO (n=10)	Control (n=10)	DMSO (n=10)	Control (n=10)	DMSO (n=10)	Control (n=10)
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	10	0	0	0	0
4	10	30	20	30	0	10
5	70	70	90	100	90	100
6	80	100	90	100	100	100
7	90	100	100	100	100	100
8	100	100	100	100	100	100

5.9 SUPEROXIDE DISMUTASE AND CATALASE

5.9.1 Background

Superoxide dismutase is a naturally occurring metalloprotein which catalyzes the dismutation of superoxide. Catalase inactivates hydrogen peroxide.

5.9.2 Methods

Superoxide dismutase (SOD) and catalase (CAT) were obtained from Sigma Chemical Company, St. Louis, MO. The toxicity of SOD and CAT was tested by intravenous administration alone and in combination with the anesthetic agent ketamine hydrochloride. Each drug was mixed in saline at a concentration of 6mg/ml. Volumes of 0.1, 0.2, and 0.3ml corresponding to 2, 4, and 6mg/kg were injected intravenously into the tail vein of three rats. Each rat then received 0.1ml ketamine intravenously and all were closely observed. Rats receiving 2 and 4mg/kg SODCAT remained under the anesthetic for approximately 10 minutes and recovered with no difficulty. The rat receiving 6mg/kg died shortly after the injection of the anesthetic. A fourth rat received 4mg/kg SODCAT intravenously and no anesthetic, and a fifth rat received 8mg/kg SODCAT with no anesthetic. Neither of these two rats exhibited any reaction to the injection. A sixth rat received 4mg/kg SODCAT intravenously followed by 0.3ml ketamine intraperitoneally. This rat was asleep from the anesthetic for approximately 25 minutes, and then recovered without difficulty.

SOD or CAT was administered intravenously to one rat at a dose of 2mg/kg every 3 minutes until a total of 34mg/kg had been infused. These rats exhibited no reaction to either drug.

Four mg/kg SOD and CAT were infused intravenously into the rat tail vein over 1.5 minutes. The rats were then given small field Maximar abdominal radiation within 15 minutes of the end of the infusion.

In a second study, either SOD or SODCAT were delivered by Harvard pump infusion in the tail vein for 5 minutes prior to abdominal radiation, during the delivery of radiation, and for approximately 5 minutes after radiation, for a total time of 20 minutes. The delivery rate was 0.078ml/min giving a total of 9.3mg/kg SOD and 3.7mg/kg CAT in a total volume of 1.4ml. These rats received 1500cGy small field Maximar abdominal radiation.

5.9.3 Results

Percent mortality for the SOD and SODCAT studies is given in table 35. Neither SOD alone or the combination of SOD and CAT improved survival.

Table 35. Percent mortality following intravenous SOD or SODCAT after 1340 or 1500cGy small field Maximar abdominal radiation.

	1340 cGY Pretreated IV		1500 cGy Continuous IV	
Day After Radiation	CONTROL (n=10)	SODCAT (n=10)	SOD (n=10)	SODCAT (n=9)
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	50	10	20	0
5	80	90	60	22
6	100	100	80	56
7	100	100	80	78
8	100	100	90	78
9	100	100	100	78
10	100	100	100	78
11	100	100	100	78
12	100	100	100	78
13	100	100	100	89
14	100	100	100	89

5.10 QUINACRINE

5.10.1 Background

Quinacrine inhibits phospholipase A and thus slows the cleavage of arachidonic acid from precursor phospholipids. The presumption is that prostaglandin production is reduced by lack of substrate.

5.10.2 Methods

Quinacrine hydrochloride was purchased from Sigma Chemical Company, St. Louis, MO. For the first study, 19 rats received 25mg/kg intraperitoneally one hour prior to 1500cGy small field abdominal radiation from the Maximar machine. Control rats received 0.5ml saline IP one hour prior to radiation. In the second study, 20 rats received 25mg/kg IP quinacrine and 48mg/kg IP Decadron one hour prior to 1500cGy small field abdominal radiation from the Maximar machine. Another 10 rats received 25mg/kg IP quinacrine plus 6mg/kg IP Celestone (betamethasone) one hour prior to 1500cGy small field abdominal radiation from the General Electric Maximar machine.

5.10.3 Results

Survival results for the first study are given in table 36. At the end of the study, 68% of the rats receiving quinacrine had died while 95% of the control rats died. This represented a significant decrease in mortality (P=0.015).

Table 36. Percent mortality for control rats and rats receiving 25mg/kg intraperitoneal quinacrine one hour prior to 1500cGy abdominal radiation from the Maximar machine.

Day After Radiation	Control (n=19)	Quinacrine (n=19)
1	0	0
2	0	0
3	0	0
4	0	0
5	21	0
6	47	16
7	58	32
8	74	42
9	74	58
10	79	63
11	84	63
12	89	63
13	89	68
14	95	68

This observation of improved mortality led us to try using a combination of quinacrine plus either Decadron or Celestone in the second study (see table 37). The combination of drugs resulted in 63% mortality for quinacrine plus Decadron(P=0.12), and 60% mortality for quinacrine plus Celestone compared to 79% (P=0.04) mortality for the control rats following 1500cGy abdominal radiation from the Maximar machine. Part of the difference is due to the number of animals in the drug groups.

Table 37. Percent mortality for control rats and rats receiving 25mg/kg quinacrine plus 6mg/kg Celestone IP or 48mg/kg Decadron one hour prior to 1500cGy abdominal radiation from the Maximar machine.

Days After Radiation	Control (n=19)	Quinacrine & Decadron (n=19)	Quinacrine & Celestone (n=10)
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	36	21	0
6	68	32	10
7	74	37	60
8	74	47	60
9	74	53	60
10	74	53	60
11	74	53	60
12	79	58	60
13	79	58	60
14	79	63	60

5.11 TRASYLOL

5.11.1 Background

Trasylol inhibits a variety of proteolytic enzymes including pancreatic trypsin. We have previously shown that absence of pancreatic juice from the lumen raises radiation tolerance.

5.11.2 Methods

Aprotinin (Trasylol) protease inhibitor was obtained from Sigma Chemical Company, St. Louis, MO. It had been extracted from bovine lung and was in 0.9% sterile saline with 0.9% benzyl alcohol. The solution contained 29 trypsin inhibitor units per ml. One trypsin inhibitor unit (TIU) will decrease the activity of two trypsin units by 50%. One TIU equals approximately 900 Kallikrein inhibitor units.

Two ml Trasylol was added to 198ml tap water giving 0.29 TIU/ml. This solution was given to the 10 rats for drinking water for 24 hours prior to and 3 days following 1500cGy abdominal radiation from the Maximar machine. During the 24 hours prior to radiation, the rats consumed an average of 71.4ml (SEM 3.4) which was equivalent to 20.7 TIU/rat/24 hours, or 76.7 TIU/kg/24 hours. Nine control rats received tap water for drinking water during this same period of time.

5.11.3 Results

Oral Trasylol, 76.7 TIU/kg/24 hours in drinking water did not decrease percent mortality following 1500cGy small field abdominal radiation from the Maximar machine when compared to rats receiving tap water. Nine of ten trasylol rats were dead by day 8 whereas 8 of 9 control rats were dead by day 8 (see table 38).

Table 38. Percent mortality of control rats and rats receiving oral Trasylol prior to 1500cGy Maximar abdominal radiation.

Day After Radiation	Control (n=9)	Trasylol (n=10)
1	0	0
2	0	0
3	0	0
4	11	0
5	33	40
6	67	70
7	78	80
8	89	80
9	89	90
10	89	90
11	89	90
12	89	90
13	89	90
14	89	90

SECTION 6

RADIATION OF EXTERIORIZED INTESTINE

6.1 METHODS

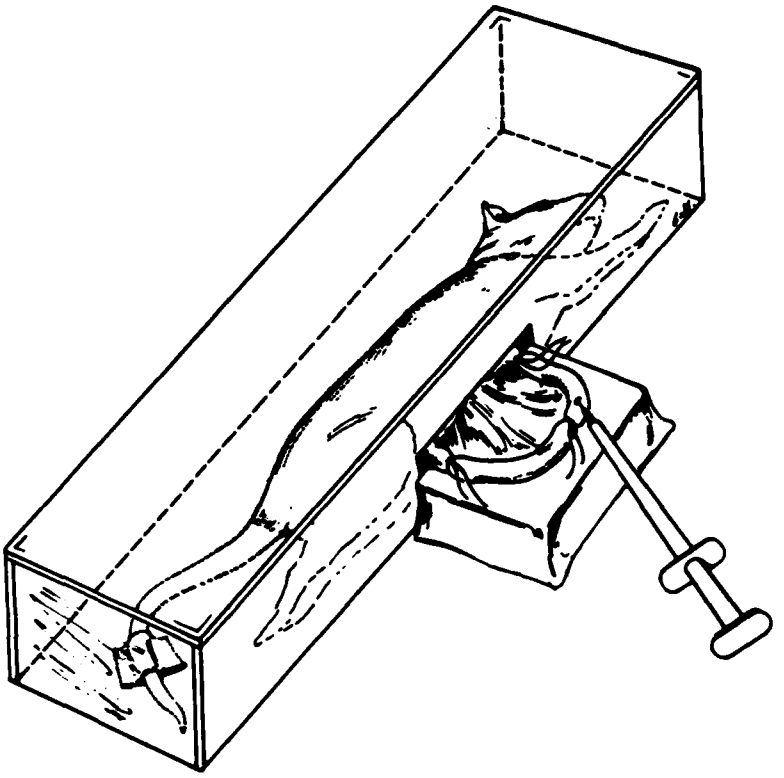
We have devised an original method for assessing the possible protective effects of lumenal agents on radiation mucosal injury using an isolated exteriorized segment of small bowel. Rats were anesthetized with 80mg/kg ketamine hydrochloride. A midline abdominal incision was made, the small bowel was exteriorized and the mid point determined. Two 5cm isolated segments were developed by means of loosely tied occluding sutures. The remainder of the bowel was returned to the abdominal cavity leaving only the segmented intestine exposed. The entire rat was placed in a lead box and the intestine to be radiated pulled through a window and placed on saline-soaked gauze. Thermistors were used to monitor body temperature, room temperature, and the temperature of the exteriorized loop.

Saline or the drug solvent was injected into the lumen of the proximal loop and the test drug into the lumen of the distal loop. The loop was covered with saline-soaked gauze and saran wrap to eliminate evaporation and maintain temperature. Figure 5 illustrates this procedure.

After bringing the exteriorized loop up to body temperature by use of an infrared lamp, radiation was delivered by the 220 KVp General Electric Maximar machine at 15 milliamps with a 0.25mm added copper filter. After radiation, the occluding sutures were removed. Marking sutures were placed in the mesentery at the sites of the occluding sutures. The intestine was replaced and the abdomen closed. Rats were allowed to recover from the anesthetic and were returned to their cages. They were allowed water and standard lab chow ad lib until the time of sacrifice.

Sacrifice was by ether overdose on post-radiation day 4 or 5, the days on which the greatest histological damage is apparent. The abdomen was opened and the previously radiated segments of intestine located by the marking sutures. The intestine was divided at the point where the occluding suture separated the saline loop from the drug loop and approximately 1cm above the proximal and below the distal marking suture. Non-radiated tissue samples were taken 10 cm above and 10 cm below the radiated bowel to serve as control specimens for each animal. All samples were processed, stained, and scored for damage as described in the General Methods section (page 15).

A second protocol involved identical radiation of the exposed intestine with sequential sacrifice of the animals on days two through ten. This experiment was aimed at measuring alterations in mucosal recovery rate by potentially protective agents.



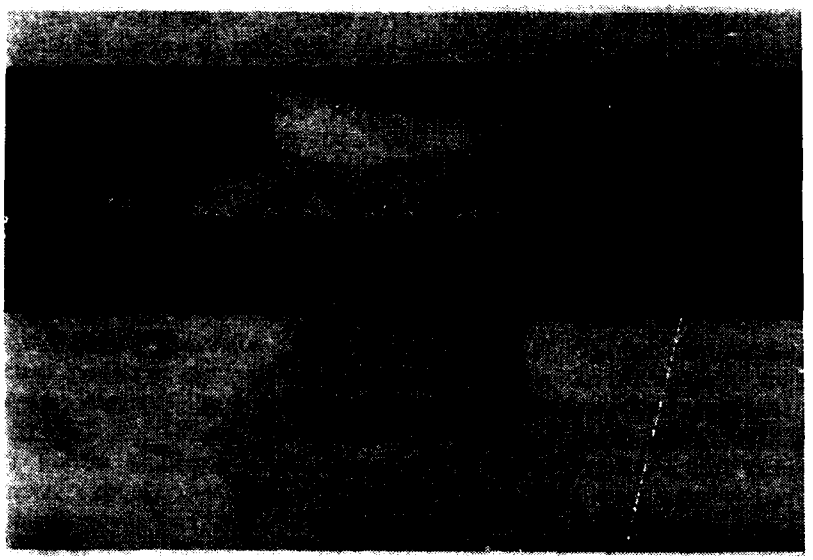


Figure 5. A 10 cm segment of intestine is exteriorized, segmented and radiated. The rest of the animal is shielded in a lead box.

6.2 PROSTAGLANDINS

Histology studies were done using the exteriorized loop model. Eight rats received 1100cGy radiation to the exteriorized intestinal loop with saline in the proximal and 10ug PGE_2 in the distal segment. They were sacrificed, five on day 4 and three on day 5. Tissue samples were taken from the proximal (saline) and distal (prostaglandin) loops, and from non-radiated proximal and distal small bowel. Intestinal samples were processed and scored for histological damage.

In six other rats, a 10cm segment of small bowel was exteriorized and divided into two sections. Saline, 0.2ml was injected into the proximal loop and 46ug in 0.2ml 16, 16 dimethyl PGE_2 was injected into the distal loop 15 minutes prior to 1100cGy radiation. Three rats were sacrificed on day 4 and three on day 5 following radiation and the radiated intestine and non-radiated control intestine evaluated histologically.

Mean mucosal heights of the saline loop, drug loop, and non-radiated small bowel for the exteriorized loop radiated rats are given in table 39. Decrease in mucosal height is associated with histological damage from radiation. For rats receiving 10ug PGE_2 and 1100 cGy, and sacrificed on post-radiation day 4, the mean mucosal height of the PGE_2 loop was 0.454mm, significantly different from 0.313mm, the mean height of the saline loop ($P<.05$). Mucus cell score for the saline loop was 3.10 +/-0.56 and for the PGE_2 loop was 2.20 +/-0.40 with a $P=.0249$.

Inflammation and vascularity were not significantly different for the saline loop and the PGE_2 loop. Rats sacrificed on post-radiation day 5 showed no significant difference between saline and drug for any of the histologic parameters of mucosa height, mucus cells, inflammation, or vascularity.

Rats receiving 16,16 dimethyl PGE_2 in the distal loop and saline in the proximal loop and 1100cGy showed no significant difference in the height of the mucosa when sacrificed on post-radiation day 4, but the mucosal height of the saline loop (0.296mm) was significantly less than the height of the drug loop (0.406) for the rats sacrificed on post-radiation day 5 ($P<.05$).

There were no significant differences between the saline loop and the 16,16 dimethyl PGE_2 loop for the other histologic parameters of inflammation, vascularity, or mucus cells.

Table 39. Mean mucosal height of intestine following PGE_2 or 16,16-dimethyl PGE_2 and 1100cGy radiation to the exteriorized loop.

Days After Radiation (n)	Drug	mm Height Saline(SEM)	mm Height Drug (SEM)	mm Height Control(SEM)
3 (1)	PGE_2	0.159	0.174	0.407
4 (5)	PGE_2	0.313 (.032)	0.454 (.040)	0.548 (.040)
4 (3)	16,16 PGE_2	0.483 (.066)	0.593 (.031)	0.536 (.058)
5 (3)	PGE_2	0.340 (.074)	0.498 (.020)	0.573 (.017)
5 (3)	16,16 PGE_2	0.253 (.033)	0.406 (.035)	0.576 (.030)
6 (1)	PGE_2	0.552	0.563	0.563
7 (1)	PGE_2	0.544	0.574	0.428
8 (1)	PGE_2	0.689	0.611	0.450
9 (1)	PGE_2	0.578	0.537	0.426
10 (1)	PGE_2	0.656	0.585	0.450

6.3 INDOMETHACIN

Twenty-two rats were prepared as described above. Indomethacin, 0.8mg in 0.2ml saline was injected in the distal segment and 0.2ml saline was injected into the proximal exteriorized segment. All rats received 1100cGy radiation from the Maximar machine. Eleven rats were sacrificed on day 4, and 12 rats were sacrificed, one each on post radiation days 1 through 12. Tissues samples were removed, prepared, and evaluated as described above. Histology results for the eleven rats sacrificed on day 4 are given in table 40.

Table 40. Mean histology values for radiated loop injected with 0.8mg indomethacin or 0.2ml saline prior to 1100cGy Maximar radiation, sacrificed on day 4.

	Indocin	Saline	P
mmMucosa Ht	0.433(.03)	0.292(.24)	<0.001
Vascularity	2.9(.25)	3.1(.21)	
Inflammation	2.9(.21)	3.3(.19)	
Mucus Cells	3.1(.26)	3.5(.21)	

During the days of mucosal recovery (five through eight), Indocin pretreated mucosa was thicker and restoring more rapidly than saline mucosa. Mucosa height measurements for the rats sacrificed sequentially days 1 through 12 are given in figure 6.

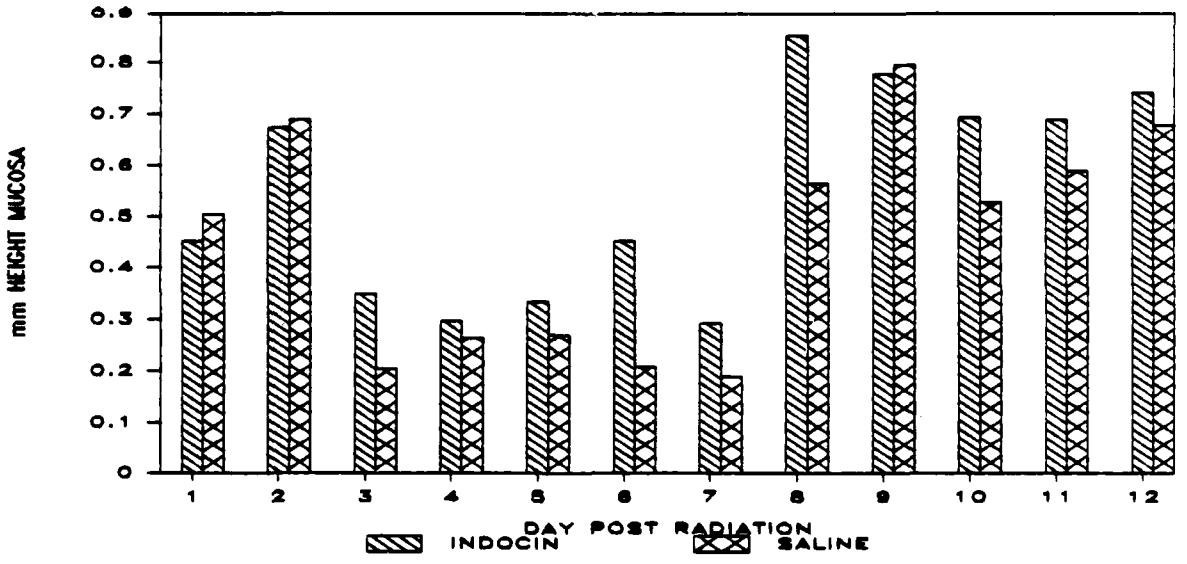


Figure 6. Mucosa height for saline and indomethacin pre-treated intestine on days 1 through 12 after 1100cGy X radiation.

6.4 ASPIRIN

Four loops were pretreated with lumenal aspirin (180 mg/ml). The animals were sacrificed four days later and intestine evaluated histologically. In each instance, mucosal height was greater with aspirin pretreatment. The control was shielded intestine from the same animal. Mean mucosa height for the aspirin loop (0.395mm +/-.015) was significantly greater than mean height for the saline loop (0.281 +/-0.33). A Student's t test for paired data gave a P=.015 (see table 41). There were no differences between inflammation, vascularity, or mucus cells for the saline segment compared to the aspirin segment. figure 7.

Table 41. Histology for control,saline and aspirin pre-treated intestine day 4 after 1100cGy x-radiation.

	CONTROL	SALINE (SEM)	ASPIRIN (SEM)
mm Mucosa Height	0.556 (.023)	0.281 (.330)	*0.395 (.015)
Vascularity	2.57 (.20)	4.00 - -	4.00 - -
Inflammation	2.00 - -	3.50 (.29)	3.50 (.29)
Mucus Cells	0.00 - -	3.25 (.25)	3.00 - -

*P=0.015 compared to saline.

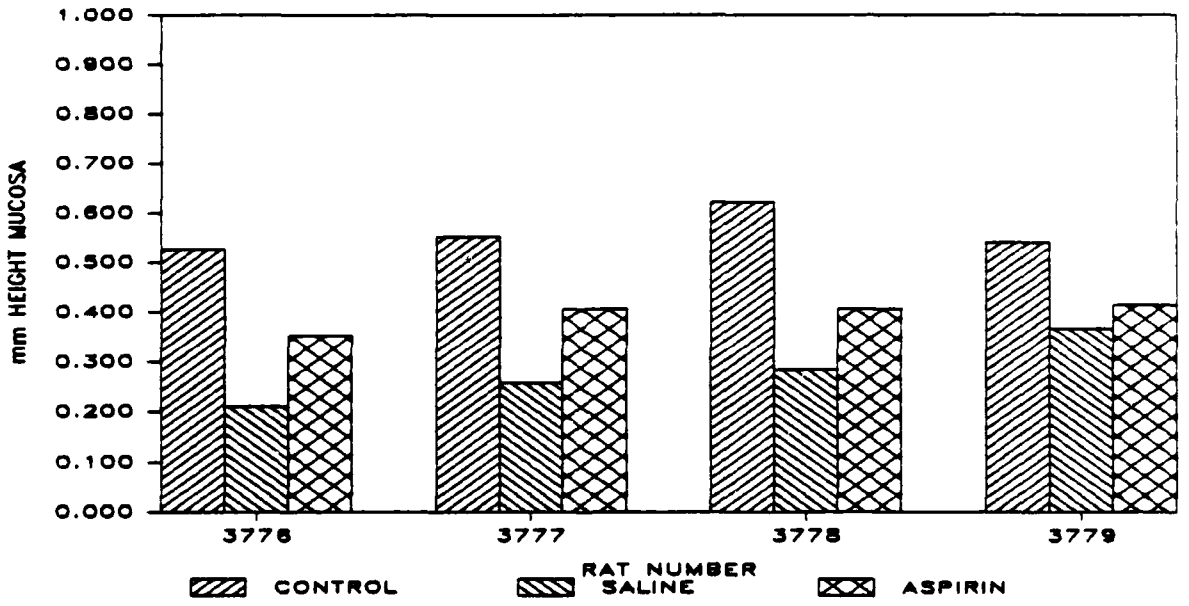


Figure 7. Mucosa height for control, saline, and aspirin pre-treated intestine day 4 after 1100cGy X-radiation.

6.5 TRASYLOL

Trasylol is an antiprotease trypsin inhibitor. Lumenal trasylol at the time of radiation seemed to enhance mucosal preservation - regeneration. The mean mucosa height for the Trasylol exteriorized loop five days after radiation was 0.438mm (SEM .039) and for the saline loop was 0.251mm(SEM .029), and a Student's t test for paired data gave P=.013. There were no significant differences between the saline and Trasylol loop damage scores for inflammation and vascularity. The mucus cell score for Trasylol was 3.00 compared to 4.00 for saline, but P values could not be calculated because there was no SEM. This supports our previous observation that the intestine devoid of pancreatic enzymes sustains less radiation damage than normal. Figure 8 shows mean mucosa height for the control, saline, and Trasylol segments of intestine.

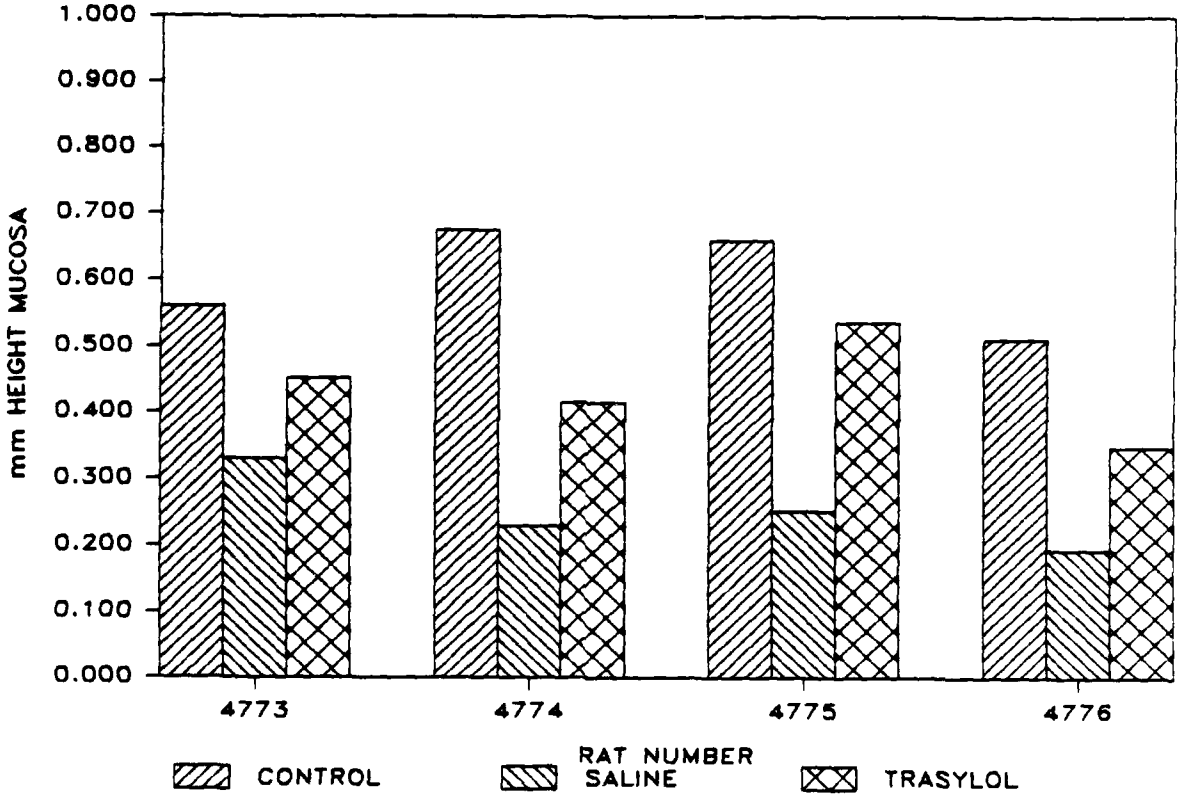


Figure 8. Mucosa height for control, saline, and Trasylol pre-treated intestine day 5 after 1100cGy X radiation.

6.6 CHOLESTYRAMINE

Pretreatment with lumenal cholestyramine, a bile salt binder, also seemed to enhance mucosal height following radiation, consistent with our previous observations with respect to the bile free intestine. For 4 rats sacrificed on day 5, the cholestyramine loop mean mucosa height was 0.496mm (SEM .059) and the saline loop mean mucosal height was 0.260mm (SEM .035, P=0.04). In two rats sacrificed on day 4, the cholestyramine treated segment showed a mean mucosa height of 0.493cm (SEM .034); the saline segment mean mucosa height was 0.295mm (SEM .080). While these measurements for the day 4 tissues indicate a greater height in the cholestyramine mucosa, they failed to reach statistical significance. The results for the day 5 animals are illustrated in figure 9.

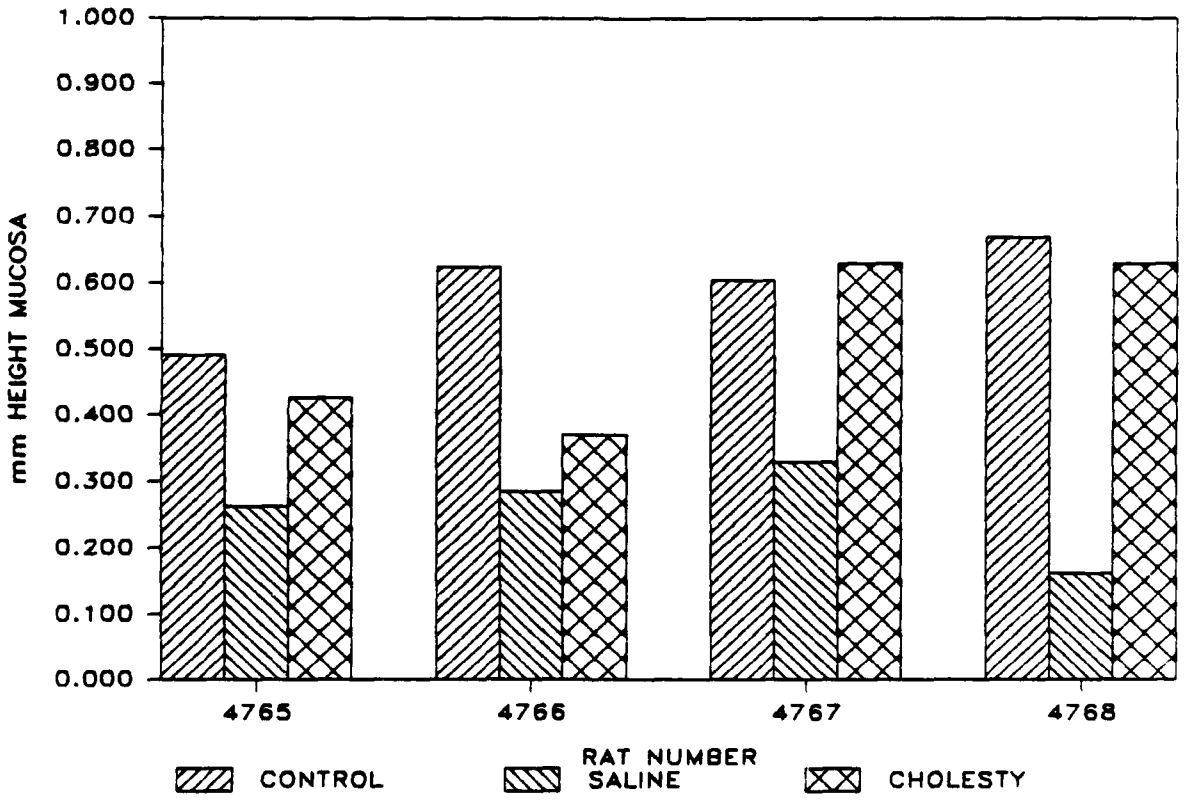


Figure 9. Mucosa height for control, saline, and cholestyramine pre-treated intestine day 5 after 1100cGy X radiation.

A second group of ten rats received the same cholestyramine pre-treatment as above, and were sacrificed two each on post-radiation days 2, 4, 6, 8, and 10. Mucosa height measurements for these animals are illustrated in figure 10.

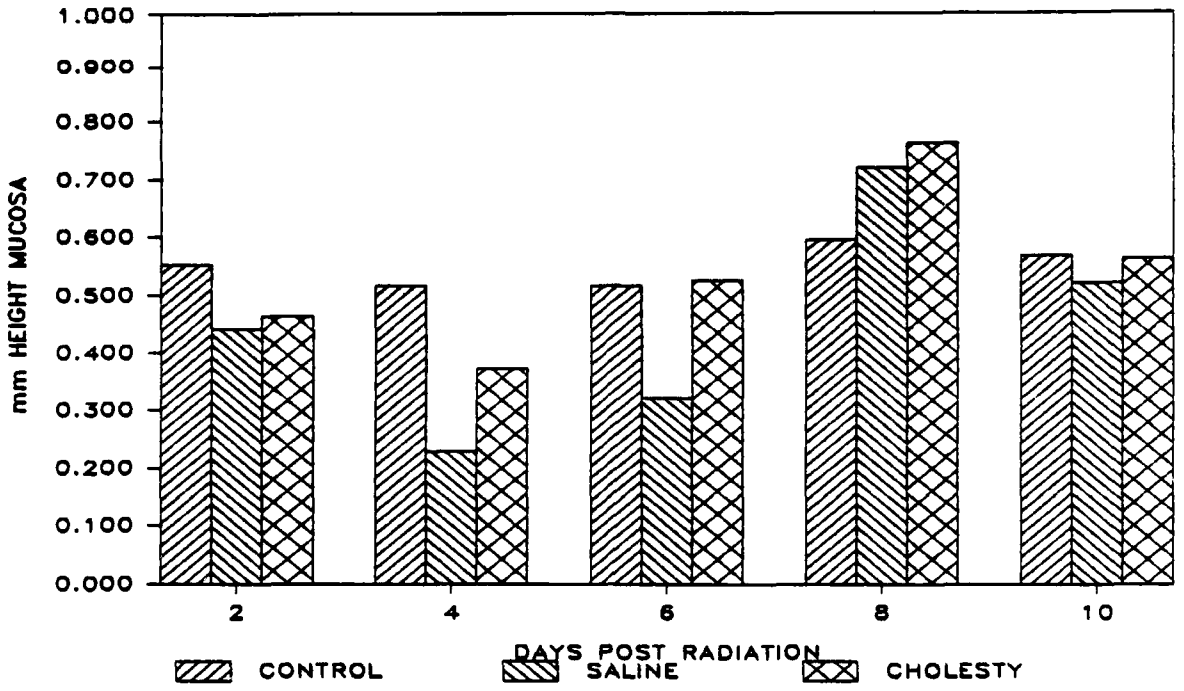


Figure 10. Mucosa height for saline and cholestyramine pre-treated intestine on days 2, 4, 6, 8, and 10 after 1100cGy X radiation.

6.7 ARACHIDONIC ACID

This is the precursor of prostaglandins. Multiple studies have shown that this agent is cytoprotective for the stomach. Five rats received 0.2ml of 120mM arachidonic acid in 5mM Pluronic F-68 (Fluka Chemical Company) in the exteriorized segment prior to 1100cGy radiation and sacrifice on day 5. In this study 5mM Pluronic was injected into the proximal segment of the exteriorized intestine. The mean mucosal height of the Pluronic loop was 0.344mm (SEM .043) while the mean arachidonic acid mucosal height was 0.333mm (SEM .029). This difference was not significant. There was also no significant difference between arachidonic acid segments and Pluronic segments for inflammation, vascularity, or mucus cells.

6.8 ALLOPURINOL

Intestinal loops were exteriorized in 2 rats and allopurinol was injected into the distal segment and saline into the proximal prior to radiation. The rats were sacrificed on day 4. Mean mucosal height of the allopurinol loop was 0.330mm (SEM .011) and mean mucosal height of the saline loop was not significantly different, 0.289mm (SEM .041). There was no significant difference between the saline segment and the allopurinol segment for vascularity, inflammation, or mucus cells.

6.9 STEROIDS

Dexamethasone or betamethasone was injected into the rat exteriorized loop prior to 1100cGy radiation. Rats were sacrificed on day 3, 4, or 5, and tissues evaluated histologically. While the results seem to indicate some mucosal protection there were not sufficient data to evaluate the measurements statistically. Results are summarized in table 42.

Table 42. Histology of dexamethasone or betamethasone in the exteriorized loop prior to 1100cGy Maximar radiation.

DRUG	# RATS	DAY	DRUG MUCOSA mmHEIGHT	(SEM)	SALINE MUCOSA mmHEIGHT	(SEM)
Betamethasone	2	3	0.417	.006	0.261	.050
Dexamethasone	1	4	0.578	- -	0.256	- -
Betamethasone	1	5	0.585	- -	0.244	- -

6.10 SUPEROXIDE DISMUTASE

Superoxide dismutase was injected into the distal exteriorized segment and saline into the proximal segment of 2 rats prior to 1100cGy radiation. The rats were sacrificed on day 4.

Histological tissue evaluation showed for two rats, the mean mucosal height was 0.571mm +/-.015 for the superoxide loop compared to 0.467mm +/-.076 for the saline loop. This difference was not statistically significant. There was also no significant difference between the two segments for inflammation, vascularity, or mucus cells.

6.11 DIMETHYL SULFOXIDE

Seven rats received DMSO in the distal segment and saline in the proximal segment 15 minutes before radiation and were sacrificed four on day 4 and three on day 5. On post-radiation day 4, the mean mucosal height of the DMSO loop was 0.482mm (SEM .047) and of the saline loop was 0.294mm (SEM .034), giving a P=.014. The rats sacrificed on day 5 also showed significantly greater mucosa height for the DMSO compared to the saline loop, 0.453 +/-.014 and 0.245m+/-.052 respectively. Measurements for inflammation, mucus cells, and vascularity showed no difference between the saline segment and the DMSO segment. These results are summarized in table 43.

Table 43. Histology of DMSO in exteriorized intestine prior to 1100cGy X radiation.

DAY	n	SALINE mmHEIGHT(SEM)	D M S O mmHEIGHT(SEM)	
4	4	0.294 (.034)	0.482 (.047)	P=.014
5	3	0.245 (.052)	0.453 (.014)	P=.083

SECTION 7

CONCLUSIONS

1) Radiation of the intestine did not enhance prostaglandin synthesis.
2) Pharmacologic manipulations of prostaglandins do not alter mortality following abdominal radiation. From these experiments there was no evidence that prostaglandins played a major role in intestinal injury.
3) Intestinal radiation does not cause enteropooling.
4) Pharmacologic manipulation of arachidonic metabolism does not alter hematopoietic suppression. None of the underlying hypothesis were supported by our experimental results.

Systemically administered free radical inhibitors or scavengers do not alter mortality after abdominal radiation. One could not safely conclude from these latter observations that free radical generation does not play a role in intestinal injury because the agents may not have reached the sight of the free radical reactions. However, one could conclude that none of the agents had a potential practical applications in this context.

There were marked and significant protective effects observed for a variety of agents placed in the lumen shortly before radiation of isolated intestinal segments.

SECTION 8

RECOMMENDATIONS

We suggest that additional studies should be performed regarding the effects of lumenal agents on radiation injury to the intestine. Such investigation could open the way for the use of oral drugs for prophylaxis against the intestinal syndrome and intestinal death.

SECTION 9

LIST OF REFERENCES

1. Berthrong M, Fajardo LF: Radiation Injury in Surgical Pathology, Part II. Alimentary tract. *Am J Surg Path 5(2)*:153-178. 1981.

2. Beubler E, Juan H: PGE release, Blood Flow and Transmucosal Water Movement After Mechanical Stimulation of the Rat Jejunal Mucosa. *Arch. Pharm. 305*:91-95, 1978.

3. Borowska A, Sierakowski A, Mackowiak J, and Wisniewski K: Prostaglandin-like Activity in Small Intestine and Postirradiation Gastrointestinal Syndrome. *Experientia 35*:1368-1370, 1979.

4. Caldwell B, Burstein S, Brock W, and Speroff L. Radioimmunoassay of the F Prostaglandins. *J. Clin. Endocrin. 33*:171, 1971.

5. Cashin CH, Dawson W, Kitchen EA: The Pharmacology of Benoxaprofen (2-[4-Chlorophenyl]-a-Menthyl-5-Benzoxazole Acetic Acid), LRCL 3794, a New Compound With Anti-Inflammatory Activity Apparently Unrelated to Inhibition of Prostaglandin Synthesis. *J. Pharm. Pharmac. 29*:330-336, 1977.

6. Clinical Radiation Pathology ed. by Rubin and Casarett, W.B. Saunders, Philadelphia, 1986.

7. Cox JW, Rutecki GW, Francisco LL, and Ferris TF: Studies of the Effects of Essential Fatty Acid Deficiency in the Rat. *Circ. Res. 51*:694-702, 1982.

8. Cronen PW, Nagaraj HS, Janik JS, Groff DB, Passmore JC, and Hock CE: Effect of Indomethacin on Mesenteric Circulation in Mongrel Dogs. *J. Ped. Surg. 17(5)*:474-478, 1982.

9. Daburon F, Villiers PA, Fagniez PL, Hay JM, Tricaud Y, and Bourhoven D: Research on the Therapeutics of Intense Abdominal Irradiations in Pigs. *Radiat. Environ. Biophys 17*:67-83, 1979.

10. Dunjic A, Maisin J, Maladague P, Maisin H: Incidence of Mortality and Dose-Response Relationship Following Partial Body X-irradiation of the Rat. *Radiation Research 12*:155-166, 1960.

11. Eisen V and Walker DI: Effect of Ionizing Radiation of Prostaglandin-like Activity in Tissues. *Br. J. Pharmac. 57*:527-532, 1976.

12. Ford-Hutchinson AW, Walker JR, Connor NS, Oliver AM, and Smith MJH: Separate Anti-inflammatory Effects of Indomethacin, Flurbiprofen and Benoxaprofen. *J. Pharm.Pharmac. 29*:372-373, 1977.

13. Granger DN, Rutili G, McCord JM: Superoxide Radicals in Feline Intestinal Ischemia. *Gastroenterology 81*:22-29, 1981.

14. Greenberg RN, Murad F, Chang B, Robertson DC, and Guerrant RL: Inhibition of *Escherichia coli* Heat-Stable Enterotoxin by Indomethacin and Chlorpromazine. *Infection and Immunity 29(3)*:908-913, 1980.

15. Hagemann RF, and Lesher, S: Intestinal Crypt Survival and Total and Per Crypt Levels of Proliferative Cellularity Following Irradiation: Age Response and Animal Lethality. *Radiation Research 47*:159-167, 1971.

16. Hanson WR. International Conference on Prostaglandin and Lipid Metabolism in Radiation Injury. Bethesda, Md. Oct, 1986.

17. Kimberg D, Field M, Johnson J, Henderson A, and Gershon E: Stimulation of Intestinal Mucosal Adenyl Cyclase by Cholera Enterotoxin and Prostaglandins. *J. Clin. Invest. 50*:1218-1230, 1971.

18. Kimberg DV, Field M, Gershon E, and Henderson A: Effects of Prostaglandins and Cholera Enterotoxin on Intestinal Mucosal Cyclic AMP Accumulation. *J. Clin. Invest. 53*:941-949, 1974.

19. Knapp HR, Oelz O, Sweetman BJ, and Oates JA: Synthesis and Metabolism of Prostaglandins EF_{2a} and D_2 by the Rat Gastrointestinal Tract. Stimulation by a Hypertonic Environment in Vitro. *Prostaglandins 15*:751-757, 1978.

20. "Mechanism of Intestinal Radiation Death" from *Gastrointestinal Radiation Injury.* Edited by Sullivan. *Excerpta Medica Foundation*, 1968, pp. 353-373.

21. Lappenbusch W. On the Mechanism of Radioprotective Action of Dimethyl sulfoxide. *Radiat Res. 46*:279-289, 1971.

22. Matsuzawa T, Wilson R. The Intestinal Mucosa of Germfree Mice After Whole-Body X Irradiation with 3 Kiloroentgens. *Radiat. Res. 25*:15-24, 1965.

23. Mennie AT, Dalley V, Dineen L and Collier H: Treatment of Radiation Induced Gastrointestinal Distress with Acetylsalicylate. *Lancet 2*:942-943, 1975.

24. Mulholland MW, Levitt SH, Song CW, Potish RA, and Delaney JP: The Role of Luminal Contents in Radiation Enteritis. *Cancer 54*:2396-2402, 1984.

25. Northway MG, Bennett A, Carroll M, Feldman MS, Mamel JJ, Lipshitz HI, Swarc IA, and Eastwood G: Comparative Effects of Anti-inflammatory Agents in Radiotherapy on Normal Esophagus and Tumors in Animals. *Gastroenterology 78*:1229, 1980.

26. Northway MG, and Castell DO: "Evidence in Suppport of an Injurious Effect of Prostaglandins on Gastrointestinal Mucosa", Chap. 16, *Gastrointestinal Mucosal Cell Injury and Protection.* ed. by John Harmon, Williams & Wilkins, Baltimore, 1981.

27. Northway MG, Lipshitz HI, Osborne BM, Feldman MS, Mamel JJ, West JH and Swarc IA: Radiation Esophagitis in the Opossum: Radioprotection with Indomethacin. *Gastroenterology 78*:883-892, 1980.

28. Osborn JW, Prasad KN, Aimmerman GR: Changes in the Rat Intestine After X-irradiation of the Exteriorized Short Segments of Ileum. *Radiation Research 43*:131-142, 1970.

29. Pawlik W, Shepherd AP, and Jacobson ED: Effects of Vasoactive Agents on Intestinal Oxygen Consumption and Blood Flow in Dogs. *J. Clin. Invest. 56*:474-490, 1975.

30. Perry MJ, Randall MJ, Hawkeswook E, Cross PE, and Dickinson RP: Enhanced Production of Prostacyclin in Blood After Treatment with Selective Thromboxane Synthetase Inhibitor, UK-38,485. *Brit. J. Pharmacol. 77*:547P, 1982.

31. Poulakos L, and Osborne JW: The Kinetics of Cellular Recovery in Locally X-irradiated Rat Ileum. *Radiation Research 47*:159-167, 1971.

32. Quastler H: The Nature of Intestinal Radiation Death. *Radiation Research 4*:303-320, 1956.

33. Robert A: "Prostaglandins and the Gastrointestinal Tract" from *Physiology of the Gastrointestinal Tract,* ed. by Leonard R. Johnson, Raven Press, New York, 1981.

34. Robert A, Nezamis JE, Lancaster C, Hanchir AJ, and Klepper MS: Enteropooling Assay: A Test for Diarrhea Produced by Prostaglandins. *Prostaglandins 11*:809-828, 1976.

35. Samuelsson B, Paoletti R: The Leukotrienes: An Introduction, from *Leukotrienes and Other Lipoxygenase Products,* 1-17, Raven Press, New York, 1982.

36. Smith PL, Blumber JG, Stoff JS, and Field M: Antisecretory Effects of Indomethacin on Rabbit Ileal Mucosa in Vitro. *Gastroenterology 80*:356-365, 1981.

37. Smith S, Grisham M, Manci E, Granger D, Krietys P: Gastric Mucosal Injury in the Rat: Role of Iron and Xanthine Oxydase. *Gastroent 92*:950-956,1987.

38. Spies C, Schultz KD, Schultz G: Inhibitory Effects of Mepacrine and Eicosatetraynoic Acid on Cyclic GMP Elevations Caused by Calcium and Hormonal Factors in Rat Ductus Deferens. *Nauyn-Schmiedeberg's Arch. Pharm. 311*:71-77, 1980.

39. Steel LK, and Catravas GN: Radiation-induced Changes in Production of Prostaglandins F_{2a}, D, and Thromboxane B_2 in Lung Airways of Guinea Pigs. *Rad. Res. 94*:156-165, 1983.

40. Steel LK, Sweedler IK, and Catravas GN: Effects of ^{60}Co Radiation of Synthesis of Prostaglandins F_{2a}, E, and Thromboxane B_2 in Lung Airways of Guinea Pigs. *Rad. Res. 94*:156-165, 1983.

41. Thomas DD, and Knoop FC: The Effect of Calcium and Prostaglandin Inhibitors on the Intestinal Fluid Response to Heat-Stable Enterotoxin of *Escherichia coli.* *J. Infect. Dis. 145(2)*:141-147, 1982.

42. Uribe A, Johansson C, Rubio C, and Arndt J: Effects of 16, 16 Dimethyl Prostaglandin E_2 on Irradiation Damage of the Small Intestine. *Acta Radiologica Oncology 23*:349-352, 1984.

43. Venuto RC, O'Dorisio T, Stein, JH, Ferris TF: Uterine Prostaglandin E Secretion and Uterine Blood Flow In the Pregnant Rabbit. *J. Clin. Invest. 55*:193-197, 1975.

44. Wald A, Gotterer GS, Rajendra GR, Turjman NA and Hendrix TR: Effect of Indomethacin on Cholera Induced Fluid Movement, Unidirectional Sodium Fluxes, and Intestinal cAMP. *Gastroenterology 72*:106-110, 1977.

45. Withers HR: Regeneration of Intestinal Mucosa After Irradiation. *Cancer 28*:75-81, 1971.

46. Yagasaki O, Suzuki H, Sohji Y: Effects of Loperamide on Acetylcholine and Prostaglandin Release From Isolated Guinea Pig Ileum. *Japan. J. Pharmacol. 28*:873-882, 1978.

47. Zusman RM, Caldwell BV, Speroff L, and Behrman H: Radioimmunoassy of the A Prostaglandins. *Prostaglandins 2*:41, 1972.

DISTRIBUTION LIST

DEPARTMENT OF DEFENSE

ARMED FORCES RADIOBIOLOGY RSCH INST
10CYS ATTN: MRCO MAJ LUCKETT

ASSISTANT SECRETARY OF DEFENSE
HEALTH AFFAIRS
ATTN: INTERNATIONAL ACTIVITIES

ASSISTANT TO THE SECRETARY OF DEFENSE
ATTN: ROOM 3E 1074

DEFENSE INTELLIGENCE AGENCY
2 CYS ATTN: RTS-2B

DEFENSE NUCLEAR AGENCY
4 CYS ATTN: TITL

DEFENSE TECH INFO CENTER
12CYS ATTN: DD

DEPUTY UNDER SECRETARY OF DEFENSE
ATTN: ENVIRONMENTAL & LIFE SCI

FIELD COMMAND DEFENSE NUCLEAR AGENCY
ATTN: FCP/FCPF

INTERSERVICE NUCLEAR WEAPONS SCHOOL
ATTN: RH

DEPARTMENT OF THE ARMY

ARMED FORCES INSTITUTE OF PATHOLOGY
ATTN: RADIOLOGIC PATHOLOGY DEPT

DIRECTORATE OF COMBAT DEVELOPMENT
ATTN: ATSA-COM-L(NBC)

LETTERMAN ARMY INSTITUTE OF RESEARCH
ATTN: SGRD-ULV-R

SURGEON GENERAL
ATTN: AAFJML
ATTN: MEDDH-N

U S ARMY ACADEMY OF HEALTH SCIENCES
ATTN: HSHA-CDD

U S ARMY NATICK RSCH DEV & ENGRG CTR
ATTN: STRNC-S

U S ARMY NUCLEAR & CHEM AGENCY
ATTN: MONA-NU

WALTER REED ARMY INSTITUTE OF RSCH
ATTN: DIV OF EXPERIMENTAL THERAPEUTICS

DEPARTMENT OF THE NAVY

BUREAU OF MEDICINE & SURGERY
ATTN: CODE 71

NAVAL AEROSPACE MEDICAL INSTITUTE
ATTN: ANIMAL BEHAVIORAL SCI BR

NAVAL AEROSPACE MED RESEARCH LAB
ATTN: COMMANDING OFFICER

NAVAL MEDICAL COMMAND
ATTN: MEDCOM-21

NAVAL MEDICAL RSCH & DEV. COMMAND
2 CYS ATTN: (CODE 40C), NMCNCR

OFFICE OF NAVAL RESEARCH
ATTN: CODE 441

RADIATION HEALTH OFFICER
ATTN: USS SIMON LAKE

DEPARTMENT OF THE AIR FORCE

BOLLING AFB
ATTN: AF/SGPT
ATTN: HQ USAF/SGES

U S AIR FORCE ACADEMY
ATTN: HQ USAFA/DFBL

USAF OCCUPATIONAL & ENV HEALTH LAB
ATTN: OEHL/RZI

USAF SCHOOL OF AEROSPACE MEDICINE
ATTN: AEROSPACE MEDICAL DIV
ATTN: CH, RADIOBIOLOGY DIV
ATTN: USAFSAM/RZB

DEPARTMENT OF ENERGY

ARGONNE NATIONAL LABORATORY
ATTN: REPORT SECTION

BROOKHAVEN NATIONAL LABORATORY
ATTN: REPORTS SECTIONS
ATTN: RESEARCH LIBRARY

DEPARTMENT OF ENERGY
ATTN: DIR OHER-ER 70

LAWRENCE BERKELEY NATIONAL LAB
ATTN: LIBRARY

LAWRENCE LIVERMORE NATIONAL LAB
ATTN: TECH INFO DIV LIB

LOS ALAMOS NATIONAL LABORATORY
ATTN: REPORT LIBRARY

LOVELACE BIOMEDICAL &
ENVIRONMENTAL RSCH INSTITUTE, INC.
ATTN: DOCUMENT LIBRARY

OTHER GOVERNMENT

CENTRAL INTELLIGENCE AGENCY
ATTN: OFFICE OF MED SERVICES

DIVISION OF LIFE SCIENCES
9CYS ATTN: DIV OF LIFE SCIENCES

GPO-CONSIGNED BRANCH
17CYS ATTN: CONSIGNED STOCK

LIBRARY OF CONGRESS
ATTN: EXCHANGE AND GIFT DIV

NATIONAL LIBRARY OF MEDICINE, NIH
ATTN: OFC OF PUBS AND INQUIRIES

NUCLEAR REGULATORY COMMISSION
ATTN: LIBRARY

PROJ OFFICER FOR RADIOLOGICAL MODIFIERS
ATTN: NIH-NCI-DCT-CTEP-RDB-BTSG

U S GOVERNMENT PRINTING OFFICE
ATTN: RM A-150

U S PUBLIC HEALTH SERVICE
ATTN: DIV OF BIOLOGICAL EFFECTS

U S PUBLIC HEALTH SERVICE
ATTN: NE RADIOLOG HEALTH LAB

UNIVERSITY OF MINNESOTA MED SCHOOL
4 CYS ATTN: DR J DELANEY

DEPARTMENT OF DEFENSE CONTRACTORS

KAMAN SCIENCES CORP
ATTN: DASIAC

KAMAN TEMPO
ATTN: DASIAC

PACIFIC-SIERRA RESEARCH CORP
ATTN: H BRODE

FOREIGN

NBC DEFENSE RSCH AND DEVELOPMENT
ATTN: WWDBW AB

SERIAL ACQUISITIONS (EXCHANGE)
ATTN: BRITISH LIBRARY

DIRECTORY OF OTHER

CALIFORNIA, UNIVERSITY OF DAVIS
ATTN: RADIOBIOLOGY LAB

OREGON STATE UNIVERSITY
ATTN: DEPT BIOCHEMISTRY & BIOPHYSICS

RADIOISOTOPE LABORATORY
ATTN: RADIOISOTOPE LABORATORY

ROCHESTER UNIV MEDICAL CTR
ATTN: RBB LIBRARY